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CONTENTS

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COMPARATIVE DEVELOPMENT OF TWO WHEAT VARIETIES UNDER VARYING MOISTURE SUPPLY[1]

BY J. W. HOPKINS[2]

Abstract

Two varieties of wheat, Lutescens and a cross of Reward × Caesium, were both grown in the greenhouse at four levels of soil moisture supply. The plants produced were harvested individually and subjected to structural counts, measurements, and weighings designed to illustrate certain components of growth and yield.

Significant effects of moisture supply on number, height, and weight of shoots, number of fertile heads, and weight, size, and nitrogen content of grain per plant and per shoot, differing in some cases in the two varieties, were demonstrable. The relation between the production of straw and fruiting parts per tiller also seemed to be a varietal characteristic. It is suggested that observations of this type, under field plot conditions, on a relatively small number of plants grown under controlled moisture supply, might provide useful information respecting the adaptability of different varieties of plants.

Analysis of agricultural yield through studies of the growth and development of crop plants is a problem which has received the intermittent attention of agronomists for some time. Balls and Holton in Egypt (1, 2), Engledow and his successive co-workers in England (3–5), Tincker and Jones in Wales (10), Smith in Australia (9), Immer and Stevenson in the United States (8), and Goulden and Elders in this country (6) are some of those who have dealt with one aspect or another of this subject. It would seem that information of this sort might find some application in both the genetical and agronomic phases of drought investigations, as well as in the field of crop estimation and forecasting (11). Accordingly, the small preliminary trial now to be described was conducted in the greenhouse of the National Research Laboratories, in order to provide some indications of the possibilities in this connection.

Experimental

Two varieties of spring wheat believed to differ in drought resistance were used, namely Lutescens and a cross of Reward × Caesium. Seed of these was kindly supplied by Prof. K. W. Neatby of the University of Alberta. The procedure was to grow all the experimental plants under uniform conditions until just after the initiation of tillering, when four levels of soil moisture

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were imposed and maintained until maturity. Individual plants were grown in steam-sterilized soil in 6-in. pots, each combination of variety and treatment being replicated six times. In order to reduce errors due to positional effects, the pots were arranged on the greenhouse bench in six randomized blocks, each block comprising one representative of each variety and treatment. The four soil moisture levels adopted were 30%, 27.5%, 25% and 22.5% respectively. These may be thought rather high for use with drought-resistant varieties, but it should be explained that these figures refer to the level to which the soil moisture was adjusted by weighing every three days, so that the average moisture available would in each case be below the figure indicated. Furthermore, the soil used, being a good quality potting loam, had quite a high moisture-holding capacity.

Seeding took place in January, and harvesting early in June, 1938.

Results

Each plant was harvested separately and subjected to certain structural counts, measurements and weighings. The results of these are described in the following paragraphs.

Table I shows the average number of shoots, heads and grain-bearing heads, the average weight of culms, of heads and of grain, and the average number and size of kernels per plant produced by the two varieties at the different moisture levels. It will be seen that the imposed differences in moisture supply had an effect on the production of secondary tillers, but that at all four moisture levels Reward × Caesium produced a consistently higher number of shoots per plant than did Lutescens. The situation in the case of the total number of heads produced is very similar, Reward × Caesium showing a significant excess at three of the four moisture levels. This variety likewise produced a consistently higher number of grain-bearing heads per plant.

The effect of moisture supply upon the production of straw is quite clear-cut, both varieties behaving very similarly in this respect and the differences between them being negligible. On the other hand, the weight of heads and of grain produced by Lutescens tends to be greater at high as well as at low moistures. There is no clear-cut distinction between the varieties in respect of the average number of kernels produced per plant, but the average weight per kernel is consistently higher in the case of Lutescens.

The manner in which the foregoing gross results were arrived at by the plant may now be considered in a little further detail. Table II shows certain characteristics of the average plant of each variety produced under the four moisture regimes. The influence of decreasing moisture supply in reducing culm length, particularly in the secondary tillers, is quite obvious. Effects on weight of head and on grain yield are also evident. In both varieties the yield from the first and second tillers is maintained fairly well throughout the moisture range, the third and fourth tillers show marked declines, and in the case of Lutescens the fifth disappears entirely. This is probably a practical advantage, since the production of a certain number of small and low-grade kernels is

TABLE I

AVERAGE STRUCTURAL COUNTS AND WEIGHTS PER PLANT

Average	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
No. of shoots	5.8	4.8	5.0	4.7	5.2	4.8	4.3	3.5
No. of heads	5.0	4.3	4.5	4.0	4.5	4.7	4.2	3.3
No. of grain-bearing heads	4.3	4.0	4.2	3.8	3.8	3.7	3.5	3.0
Wt. of culms, gm.	1.9	1.9	1.6	1.5	1.2	1.2	0.9	0.8
Wt. of heads, gm.	4.1	4.7	4.0	3.9	3.0	3.7	2.7	2.8
Wt. of grain, gm.	3.1	3.8	3.0	3.0	2.2	2.9	2.0	2.2
No. of kernels	107	109	103	93	75	94	71	62
Wt. per 1000 kernels, gm.	28.9	35.1	29.4	32.8	29.3	30.8	28.3	34.4

TABLE II

AVERAGE STRUCTURAL MEASUREMENTS PER TILLER

Average	Tiller No.	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
		Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
Length of culm, cm.	1	74.5	77.8	79.0	72.2	66.3	67.3	68.4	64.2
	2	71.4	69.4	70.8	65.8	59.6	60.6	54.6	53.1
	3	62.6	63.0	62.7	60.6	52.7	55.3	43.6	43.3
	4	48.8	47.6	52.1	41.4	39.4	44.8	35.1	10.1
	5	30.6	22.7	24.0	6.3	17.8	16.0	5.0	0.0
Wt. of head, gm.	1	1.07	1.44	1.06	1.21	0.82	1.17	0.93	1.22
	2	1.10	1.29	1.06	1.12	0.87	0.97	0.94	0.87
	3	1.04	1.12	0.97	1.07	0.76	0.95	0.58	0.55
	4	0.56	0.75	0.62	0.50	0.31	0.52	0.23	0.04
	5	0.23	0.10	0.16	0.01	0.08	0.08	0.01	0.00
Wt. of grain, gm.	1	0.81	1.26	0.85	1.01	0.64	0.95	0.76	1.00
	2	0.82	0.98	0.80	0.96	0.72	0.77	0.68	0.73
	3	0.81	0.93	0.78	0.83	0.64	0.77	0.52	0.48
	4	0.36	0.51	0.51	0.34	0.16	0.38	0.14	0.05
	5	0.12	0.04	0.09	0.00	0.03	0.00	0.00	0.00
No. of kernels	1	28	36	30	30	22	28	28	29
	2	30	25	27	29	24	27	24	21
	3	28	28	26	25	22	25	14	12
	4	14	15	17	10	6	12	4	1
	5	5	2	3	0	1	0	0	0

thereby avoided. The additional grain yield of Lutescens, noted in Table I, is thus mainly attributable to the superior performance of the first, second and third tillers and not, as might perhaps have been expected, to a better sustained fruiting capacity in the fourth and fifth side tillers.

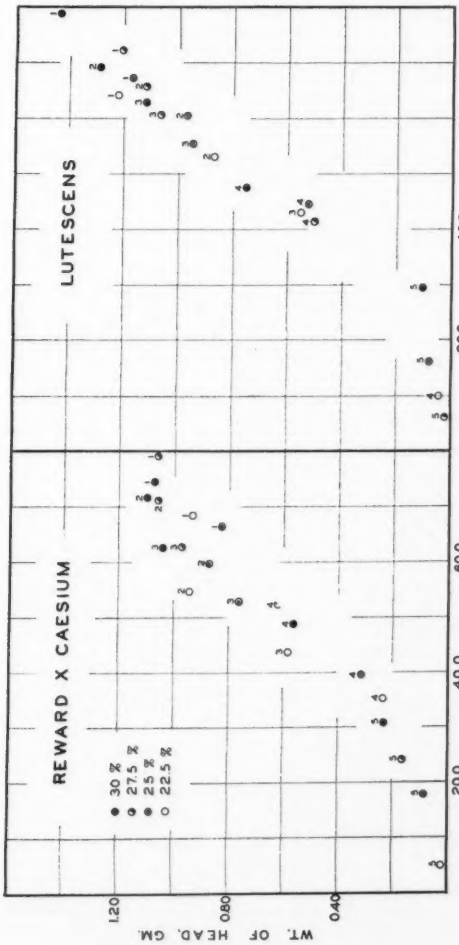

FIG. 1. *Average length of culm and weight of head of successive tillers of plants grown under four different levels of soil moisture supply. Superscripts indicate first, second, third, etc., tillers.*

Turning now to intra-varietal comparisons, Fig. 1 shows for each variety separately the average weight of the head plotted against the average length of culm of successive tillers of the plants produced under each condition of soil moisture supply. For the most part there is a fairly close relation between these two attributes, which is maintained under all four soil moistures. The

four values for the first tillers of Reward × Caesium, however, (which are distinguished in the diagram by the superscript 1) provide exceptions to this general tendency. It is thought that this anomaly may be due to the fact that the primordia from which these heads developed, being the first to be

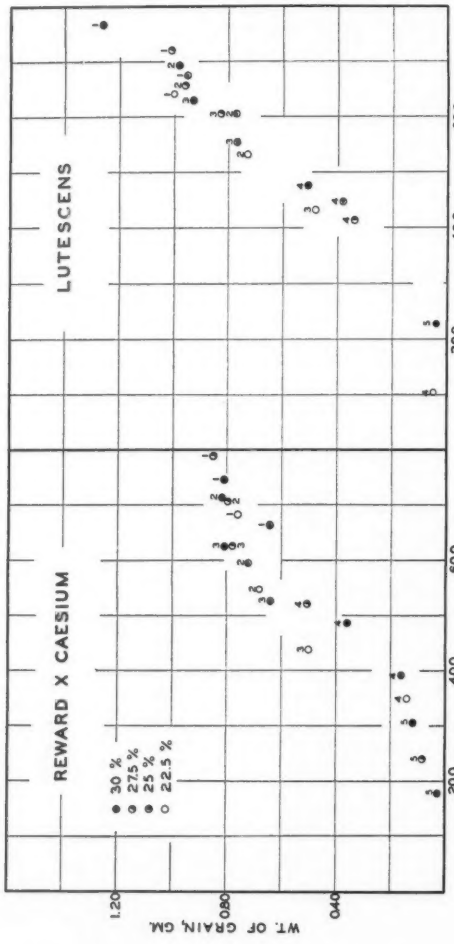

FIG. 2. *Average length of culm and weight of grain of successive tillers of plants grown under four different levels of soil moisture supply. Superscripts indicate first, second, third, etc., tillers.*

laid down, suffered from unfavourable growth conditions (chiefly low light intensity) in the greenhouse during the winter period. If the points in question are excluded, the correlation is appreciably improved. In the case of Lutescens, the relation as a whole is quite regular and the first tillers fit well into the general trend. It will be observed that the correlation in both cases is non-

linear, and that the curve for Lutescens rises more steeply. The point and degree of curvature, as well as the general slope, may therefore be varietal characteristics of some practical importance under drought conditions, since they are measures of the extent to which the production of fruiting parts is maintained when vegetative development, as indicated by straw length, is limited.

Fig. 2 shows a very similar relation between grain yield and culm length. The correlation is again curvilinear, and the results for all four moistures fall into the same general system, but the actual details of the relation would seem to be somewhat different for the two varieties. The curved form of the relation is suggestive of an example of the differential relative growth rates of organs, of the type made familiar by Huxley and termed by him "heterogony" (7).

This supposition receives some support when the foregoing data are replotted on a logarithmic scale, as is shown in Fig. 3, the approach to the required linearity being quite close, particularly in the case of Lutescens. Here also, then, there might be a possibility of distinguishing between varieties, on the basis of their relative growth gradients.

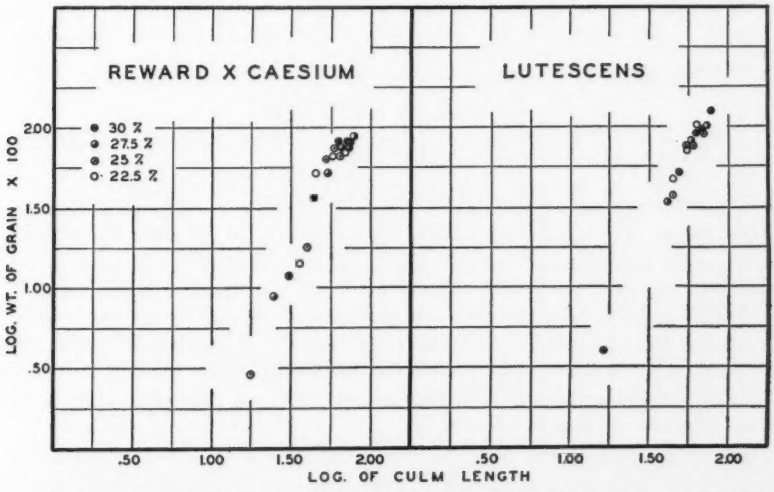

FIG. 3. *Average length of culm and weight of grain, in logarithmic units, of successive tillers of plants grown under four different levels of soil moisture.*

Differential relative growth rates may occur not only in separate organs, but also in different parts of the same organ, as is illustrated in Fig. 4. Here the average length of the top internode of the various culms of the plants at each soil moisture is plotted against the corresponding average length of the basal internodes. Once more there is a tendency towards curvilinear correlation, the length of the topmost internode increasing rather more than propor-

tionately with that of the basal ones. It may also be noted that the ratio of top to basal internode length is in general higher for Reward × Caesium than for Lutescens.

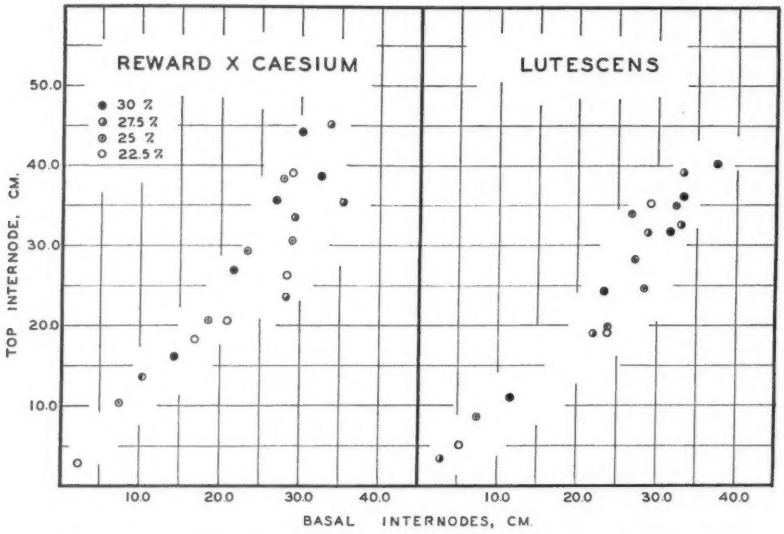

FIG. 4. *Average length of basal and top internodes of culms of successive tillers of plants grown under four different levels of soil moisture supply.*

Whilst the general trend is quite clear, there are a number of individual irregularities, and it seemed worth while to ascertain whether these were significantly associated with grain yield. It was found that the association between yield and total length of culm gave rise to a correlation coefficient of 0.948 in the case of Reward × Caesium and 0.972 in the case of Lutescens. In neither instance was this correlation significantly increased by considering the lengths of the upper and lower internodes separately.

Table III shows the results of nitrogen analyses, by the Kjeldahl method, of the grain from the individual heads.

Weighting the result for each head in proportion to its contribution to the total yield, the nitrogen content of both varieties clearly increases as soil moisture is withheld and yield reduced. From the unweighted averages of the first, second, etc., tillers it is further seen that this increase is to some extent distributed over the whole plant. In Reward × Caesium, however, the increment is more pronounced in the fourth and fifth side tillers than in the first three. In Lutescens, on the other hand, this is not the case to nearly the same extent, since the fifth tillers of this variety, even if present, were infertile at all but the highest moisture level. The results as a whole thus do not in all respects parallel those reported by Engledow and Wadham (5),

TABLE III

AVERAGE NITROGEN CONTENT OF GRAIN, AS PERCENTAGE OF DRY MATTER

Average	30% soil moisture		27.5% soil moisture		25% soil moisture		22.5% soil moisture	
	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens	Rew. × Caes.	Lutescens
Tiller No. 1	3.62	2.93	3.58	3.43	3.80	3.42	3.87	3.70
No. 2	3.54	2.84	3.54	3.17	3.73	3.41	3.54	3.64
No. 3	3.38	2.74	3.27	3.25	3.78	3.22	3.70	3.50
No. 4	2.94	2.75	3.26	3.39	4.04	3.35	4.20	3.93
No. 5	3.30	2.64	3.91	—	4.49	—	—	—
Whole plant	3.37	2.81	3.37	3.23	3.72	3.33	3.73	3.62

who found a progressive increase in the nitrogen content of the grain from successive side tillers of barley plants grown under English field conditions.

In conclusion, it should perhaps be once again pointed out that the purpose of this study was purely exploratory, and it is not suggested that the present results, secured under winter greenhouse conditions, would be duplicated in all respects in the field. It does seem reasonable to infer, however, that observations of this type, under field plot conditions, of a relatively small number of plants grown under controlled moisture supply, might provide useful information respecting the adaptability of different varieties of plants, and also respecting the morphological aspects of drought resistance in general.

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THE ACCURACY OF THE PLATING METHOD FOR ESTIMATING THE NUMBERS OF BACTERIA AND FUNGI FROM ONE DILUTION AND FROM ONE ALIQUOT OF A LABORATORY SAMPLE OF SOIL[1]

By Norman James[2] and Marjorie L. Sutherland[3]

Abstract

Six 25-gm. aliquots taken from a well-mixed laboratory sample were plated, each in six replicate dilutions with four replicate plates from each dilution, for counts of fungi. The analysis of variance shows that there are not significant differences in counts of fungi among replicate dilutions from one aliquot, but that there are among aliquots from one laboratory sample. Each replicate dilution was raised and the final dilutions plated in four replicates for counts of bacteria. The data for bacterial counts show significant differences among dilutions from one aliquot, but not among aliquot samples.

In a second experiment, one 25-gm. aliquot taken from a sample was diluted 1 : 10 and another was diluted 1 : 50. Each original dilution was raised to 1 : 5,000 in 11 replicate dilutions, which were plated in four replicates for fungi. The experiment was repeated 10 times. In this case, the data show that the 1 : 10 method of making the original dilution yields significant differences among the final dilutions and that the 1 : 50 system, which reached 1 : 5,000 in one transfer, is preferable. Each dilution was raised to 1 : 500,000 and the final dilutions were plated for bacteria in six replicates. The analysis shows that the 1 : 10 method is not reliable because of significant differences among dilutions and that the 1 : 50 method is preferable, although failing to reduce the differences to insignificance.

The 1 : 50 and 1 : 100 systems of making the original dilution were compared in Experiment 3, as well as differences among aliquot samples. A fresh sample was plated in five aliquots for each system, each aliquot in ten replicate dilutions and each dilution in four replicate plates for bacteria. The 1 : 50 system again shows significant differences among dilutions and the 1 : 100 system is not preferable. Likewise, there are significant differences among aliquot samples in each case.

In Experiment 4 all dilutions were raised from 1 : 50 original dilutions. Each trial consisted of six aliquots, raised in six replicate series of dilutions and plated in six replicate plates from each final dilution. This was repeated four times for fungal counts and six times for counts of bacteria. The analysis again shows that for fungal counts differences among dilutions are not significant, while for bacterial counts they are. Again, there are significant differences for aliquot samples in the case of both fungal and bacterial counts.

In Experiments 2, 3, and 4, the plating, pouring, piling of plates in the incubator and counting of plates were carried out in one order. The analysis shows that none of these practices adds anything significant to the error of plating.

As the errors of the sample used and of the dilution plated are significant, reliable information on the counts of bacteria, actinomyces, or fungi in a laboratory sample is not obtained by the usual procedures with one 25-gm. sample and one final dilution from it, regardless of the number of replicate plates made from the dilution. By the use of six aliquot samples with three replicate dilutions from each, and one plate for each dilution, the estimate would be based upon these three factors in about their proportionate weight.

Only by carefully designed experiments and the application of statistical methods to check the validity of the results obtained can progress be made in developing the plate method of counting bacteria or fungi in soil to a stage where it may be used for practical application to the problems of agriculture.

[1] *Manuscript received January 3, 1939.*

Contribution from the Department of Bacteriology and Animal Pathology, The University of Manitoba, with financial assistance from the National Research Council of Canada. An adaptation of section B of a thesis submitted by the senior author to the Graduate College of the Iowa State College in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in December, 1938. The complete data are in the thesis in the library of that institution.

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Introduction

At the conclusion of the plating of 504 samples of soil by the methods described in a previous paper (3), it seemed apparent that counts on samples from plots receiving one cultural or fertilizer treatment varied appreciably and did not show a consistent relation to those from plots treated differently. The 36 samples at each sampling represented six treatments replicated six times. Each plot was sampled seven times during the season. On the assumption that 42 replicate samples from plots receiving a given treatment would yield counts differing from 42 replications from similar plots that were not treated, it seemed advisable to consider the probability of there being a weakness in the technique of dilution and sampling in the laboratory. A series of replicate dilutions from one sample could be made and a statistical test applied to the data to determine whether the result from one dilution gives a reliable estimate of the population in the sample. Similarly, a series of aliquot samples could be studied to determine whether one sample is sufficient to give a reliable estimate of the population in the large sample brought to the laboratory. The analysis of variance was considered for this study.

In view of the fact that counts of bacteria and fungi in general follow the Poisson series, it may be questioned whether the analysis of variance should be applied to such data. This subject has been considered by Cochran (2), who makes the following statement, "So long as treatment responses are of the order of 50% or under, and the standard error per plot is under 12%, there can be little wrong with the use of analysis of variance, no matter what form the data analyzed may take". When the analysis is applied to data obtained for the improvement of technique there appears to be no reason for making adjustments of the data before the analysis is made. The variation among means for dilutions in the data presented frequently exceeds the 50% limit in the case of counts of bacteria plated from dilutions raised from the 1 : 10 original dilutions. When the technique is improved by using the 1 : 50 system of making the original dilutions the variation in means is below 40% in the majority of cases. The standard error for the units analyzed for variance among dilutions is of the order of about 15% for counts of bacteria and 20% for fungal counts.

The variation among replicate counts of fungi has been considered (3), and has been found to conform to expectancy on the basis of random sampling. This variation among parallel plates may be accepted arbitrarily as the error in the analysis of variance. If the variance among dilutions is larger than the error variance, there is sound reason for considering this to be a source of serious error in the method of obtaining counts by the plate technique. If, on the contrary, the variance is about proportional to the error, this may be accepted as indicating that the system of diluting adds nothing to the error. The same holds true for the aliquot sample used in making the dilution.

The case for bacteria is more complicated. The distribution of χ^2 values in the earlier studies does not conform to expectancy, and the error is large

as shown by an excess of high χ^2 values. Significant variation among dilutions or aliquot samples is even more serious than in the case of fungi, since it is compared with an error that may be larger. Fortunately, in the later studies this error is reduced and the effects of dilutions and aliquot samples are shown with more precision.

Experiment 1

Method of Procedure

A sample held in the laboratory one week was sieved and mixed thoroughly. Six 25-gm. aliquots were transferred to six 240-ml. dilution blanks and shaken as described previously (3). Each 1 : 10 dilution, obtained in this manner, was given a vigorous shaking by hand immediately before a transfer to each of six higher dilutions was made. These replicate dilutions were raised to make 36 final 1 : 5,000 dilutions representing six aliquots from one sample. Each dilution was plated in four replicates for counts of fungi.

Each dilution was raised to 1 : 200,000 and plated for counts of bacteria. Owing to a mishap one plate was lost. Accordingly, the data represent 30 dilutions from five aliquot samples.

Results

In the analysis of variance for each aliquot sample there are 5 degrees of freedom for dilution variance and 18 for error variance. For 18 degrees of freedom (the smaller mean square) and 5 degrees of freedom (the greater mean square), the 5% level of significance has an F value of 2.77 (4). The six aliquot samples have F values for dilutions as follows: 0.649, 1.20, 1.53, 1.36, 0.740 and 0.415. These are all less than the 5% level of significance.

The complete analysis of variance for the six aliquot samples follows in Table I.

TABLE I

Complete analysis of variance—fungi

	Sum of squares	D.f.	Mean square	F	5% level
Total	3928.000	143			
Aliquot samples	715.167	5	143.0332	6.00	2.30
Dilutions	636.833	30	21.2278	.89	1.56
Error	2576.000	108	23.8518		

These results suggest that for fungal counts the variance among dilutions does not add to the error of plating, while that among aliquot samples introduces serious error in the method.

A similar analysis of the data for counts of bacteria on five aliquot samples shows F values for dilutions as follows: 4.04, 5.52, 1.46, 0.435 and 2.14.

Two of these are above the 5% level of significance. The complete analysis for the five aliquots follows in Table II.

TABLE II

COMPLETE ANALYSIS OF VARIANCE—BACTERIA

	Sum of squares	D.f.	Mean square	*F*	5% level
Total	13621.9667	119			
Aliquot samples	520.0921	4	130.0230	1.61	2.47
Dilutions	5840.8746	25	233.6350	2.90	1.63
Error	7261.0000	90	80.6778		

This analysis shows that for bacterial counts the variance among dilutions is significant, while that among aliquot samples is not. Again, attention is drawn to the fact that the error for counts of bacteria is large. Accordingly, the effect of variation among dilutions undoubtedly is more serious than the result shows. Further, the finding for aliquot samples may be affected seriously by the errors of replicate plates and dilutions. These errors must be reduced to a minimum before valid comparisons for dilutions or aliquot samples can be made.

Experiment 2

This experiment was undertaken before the error of replicate plates for counts of bacteria was under proper control. Accordingly, the error variance may be large and the effect of variation among dilutions may be more serious than is shown in the data. Three points appeared worthy of further study; firstly, to substantiate the finding concerning dilutions for fungal and bacterial counts on a more comprehensive scale; secondly, to determine whether the use of a larger quantity of water in the original dilution would lessen the discrepancy among counts from different dilutions from one aliquot sample; and thirdly, to ascertain whether there was systematic error in plating. The last problem seemed important from the standpoint of reference to many minor points in technique such as: the order of plating, the order of piling plates in the incubator and the order of counting plates.

METHOD OF PROCEDURE

The soil samples were held in the laboratory up to seven days before plating. In each trial the sample was sieved and mixed thoroughly. A 25-gm. aliquot was suspended in 240 ml. of sterile water. From this 1 : 10 dilution 11 replicate series of dilutions were made, yielding 11 final 1 : 5,000 dilutions for plating for counts of fungi. Another 25-gm. sample was suspended in 1240 ml. of sterile water. From this 1 : 50 dilution 11 series of dilutions were made to give the 1 : 5,000 dilutions. Each final dilution was plated in four

replicates. Thus each trial gave 44 plates from 11 dilutions of one sample using the 1 : 10 system of making the original dilution, and another 44 plates using the 1 : 50 system. The experiment was replicated ten times.

The dilutions used for making fungal counts were raised to 1 : 500,000. These were plated for counts of bacteria in six replicates. This gave 66 plates for bacterial counts for each system of making the original dilutions. Likewise, this procedure was repeated ten times.

To introduce a large interaction variance between dilutions and replicate plates, if possible, the plates were prepared, poured, piled in the incubator and counted in systematic order. In the analysis, the interaction variance is based on columns, obtained by recording the counts in order. The number of degrees of freedom for interaction is the product of the number of degrees of freedom for dilutions and that for parallel sets of plates.

RESULTS

In the analysis of variance for each sample there are 10 degrees of freedom for dilution variance and 33 for error. For 33 and 10 degrees of freedom the F values at the 20 and 5% levels of significance are 1.465 and 2.153, respectively.

The 1 : 10 method of making the original dilutions for counts of fungi gives the following F values for dilutions in the ten replications: 1.49, 2.30, 0.880, 0.996, 1.61, 1.58, 1.59, 2.35, 0.726 and 0.648. These may be distributed and compared w[illegible]he theoretical distribution as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	5	3	2
Theoretical	8	1.5	.5

The 1 : 50 method of making the original dilutions gives the following F values for dilutions: 0.939, 0.718, 1.39, 1.98, 0.546, 2.01, 0.803, 0.655, 2.22 and 0.694. Their distribution follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	7	2	1
Theoretical	8	1.5	.5

These results appear to suggest that there are significant differences among dilutions in counts of fungi and to indicate that the 1 : 50 method of making the original dilutions is preferable.

As the experiments that follow yield further information on the interaction variance in plating, this part of the data will be considered later.

In the analysis on bacterial counts there are 10 degrees of freedom for dilution variance and 55 for error variance. For 55 and 10 degrees of freedom the F values at the 20% and 5% levels of significance are 1.42 and 2.00, respectively.

The ten F values obtained by the 1 : 10 method of making the original dilutions follow: 3.53, 3.07, 2.94, 4.16, 2.63, 2.65, 2.73, 2.15, 3.75 and 1.31, with this distribution:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	1	0	9
Theoretical	8	1.5	.5

The 1 : 50 method of making the original dilutions gives the following F values for dilutions: 1.00, 2.00, 1.28, 1.78, 0.943, 2.49, 1.68, 1.45, 1.67 and 1.72, distributed as:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	4	4	2
Theoretical	8	1.5	.5

Here, as with fungal counts, the results for the 1 : 50 method show an error for dilutions beyond that for replicate plates, but indicate a marked reduction in variation due to dilutions.

Experiment 3

The 1 : 50 method of making the original dilutions appeared to reduce the differences among dilutions. Consequently, this experiment was designed to confirm the finding, to determine the effect of further increasing the proportion of water in the original dilution, and to measure differences in aliquot samples by the improved technique.

Method of Procedure

The sample used in this study was plated on the day it was taken from the field. The counts of bacteria only were considered. The 1 : 50 and 1 : 100 methods of making the original dilutions were compared in five replications. Each consisted of ten replicate dilutions with four plates from each. This gave 200 plates for each method of making the original dilution.

Results

In this experiment there are 9 degrees of freedom for dilution variance and 30 for error. For 30 and 9 degrees of freedom the F values at the 20 and 5%

levels of significance are 1.482 and 2.225 respectively. The 1 : 50 method of making the original dilutions gives F values for dilutions of 2.37, 0.901, 1.05, 2.11 and 2.03, distributed as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	2	2	1
Theoretical	4	.75	.25

The 1 : 100 method of making the original dilutions gives F values of 1.26, 1.11, 2.87, 3.27 and 3.83, with the following distribution:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	2	0	3
Theoretical	4	.75	.25

This result suggests that the 1 : 100 method of making the original dilutions is not an improvement over the 1 : 50 method in so far as the error caused by dilution differences is concerned.

For 150 and 4 degrees of freedom for aliquot samples, the 1% level of significance has an F value of 3.44. In the 1 : 50 method, the F value for aliquot samples is 7.80; and in the 1 : 100 method, 9.64. These results indicate significant differences in counts of bacteria among aliquots taken from one sample. In this case the error of replicate plates was reduced to that of random sampling by the use of a fresh sample. Likewise, the error of dilutions was lessened appreciably by the system of diluting.

Experiment 4

This experiment was designed primarily to determine whether there are significant differences in counts of fungi and of bacteria among aliquot samples, and to obtain more data on dilution variance and on interaction variance in plating from samples of soil plated on the day obtained from the field.

Method of Procedure

All plating was carried out on dilutions prepared from 1 : 50 original dilutions. Each trial consisted of six aliquot samples. Each aliquot sample was plated in six dilutions with six replicate plates from each. This was repeated four times for fungal counts and six times for counts of bacteria.

Results

For 180 and 5 degrees of freedom for aliquot samples, the 1% level of significance has an F value of 3.122. The data for fungi give F values of 78.48, 2.09, 53.14 and 3.49. For bacteria the F values for aliquot samples are

15.46, 2.16, 6.21, 5.97, 27.16 and 19.11. From these figures it appears obvious that aliquot samples introduce a large and serious error in estimating the populations of fungi and bacteria in a sample brought to the laboratory.

When the data for dilutions are considered, the findings of previous experiments are confirmed. In the case of fungi there are 30 and 5 degrees of freedom for dilutions. For these degrees of freedom, the F values at the 20 and 5% levels of significance are 1.57 and 2.53, respectively. The distribution follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	18	4	2
Theoretical	19.2	3.6	1.2

The distribution for bacterial counts is as follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	19	7	10
Theoretical	28.8	5.4	1.8

Summary of Data for Dilution Variance

The following distributions combine the dilution variance data on the 1 : 50 system of making the original dilutions for Experiments 2 and 4 in the case of fungal counts, and for Experiments 2, 3 and 4 for counts of bacteria. The degrees of freedom available for the estimation of the effect of dilutions vary in the different experiments, but the 20 and 5% points for each experiment were determined as the F values were distributed into classes in each case. This is the best evidence at our disposal in this study on the effect of dilutions, although it cannot be taken as final proof because of the small number of values involved.

The distribution of 34 F values for fungal counts is presented first:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	6	3
Theoretical	27.2	5.1	1.7

The distribution of 51 F values for counts of bacteria follows:

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	13	13
Theoretical	40.8	7.65	2.55

Summary of Data for Systematic Error

As referred to in Experiment 2, a deliberate attempt was made in the last three experiments to introduce as large a systematic error as possible without interfering with the regular routine of plating. As one would expect the same sources of systematic error to affect the fungi and the bacteria alike, the two sets of data for distributions are considered together.

P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	92	18	8
Theoretical	94.4	17.7	5.9

In this attempt to introduce a systematic error it is apparent that the routine technique involved in large scale plating of soil samples for counts of fungi or bacteria adds nothing significant to the error.

Discussion

The variation between two dilutions from one aliquot sample or between two aliquots from one laboratory sample introduces a problem that does not appear to have been considered previously. It is obvious that the count obtained, however accurate it may be made by refinements of laboratory technique and replication of plates, represents merely an accurate count *on the dilution plated*. This limitation must be recognized. If the dilution plated is not representative of the sample in the laboratory or the soil in the field, the count has no value for the purpose intended.

The data show clearly that one dilution made by starting with 25 gm. of soil in 240 ml. of sterile water and raising it to the required dilution for plating does not provide a reliable sample for estimating the number of bacteria or fungi per gram in the 25 gm. of soil used. Variation in dilution blanks and in pipettes undoubtedly causes part of the error of dilutions in this study. Each 1 : 5,000 final dilution plated for fungi represented two consecutive dilutions of the original 1 : 10 dilution or one dilution of the 1 : 50 original dilution. Likewise, each final 1 : 500,000 dilution plated for bacteria and actinomyces represented three consecutive dilutions of the 1 : 10 original dilution and two only of the 1 : 50 original dilution. That is, the error of the dilution blanks and of pipettes used in transferring was in the ratio of 2 to 1 for fungal counts and 3 to 2 for bacterial counts, when the 1 : 10 system of obtaining the original dilutions is compared with the 1 : 50 system. The 1 : 50 system of making the original dilution proves to be superior in that it reduces differences between replicate dilutions for counts of fungi and bacteria.

An interesting comparison of the results obtained by using two consecutive dilution blanks in raising the original dilution to the one used for plating fungi and bacteria may be made. The data on analysis of variance for counts of fungi in dilutions raised from the 1 : 10 original dilutions in two consecutive

dilutions, presented in Experiment 2, show the distribution of F values in classes for F corresponding to certain P values. The theoretical number in each class is given also. A ratio may be obtained by dividing the actual by the theoretical number in each class. Similar data for counts of bacteria, in dilutions raised from the 1 : 50 original dilutions in two consecutive dilutions, may be found in the summary of data for dilution variance, and similar ratios may be obtained. The ratios follow:

	Fungi, 1 : 10 dilution			Bacteria, 1 : 50 dilution		
P	1.00–0.20	0.20–0.05	0.05–0.00	1.00–0.20	0.20–0.05	0.05–0.00
Actual	5	3	2	25	13	13
Theor.	8	1.5	.5	40.8	7.65	2.55
A/T	.625	2	4	.613	1.70	5.10

The close similarity in the ratios of actual to theoretical in the corresponding classes for the two sets of data seems to suggest that variation in dilution blanks and in pipettes is one of the main causes of the error of dilutions. When the 1 : 50 method of making the original dilution is used, one dilution blank only is required to raise to the 1 : 5,000 dilution used for plating. In the data for fungi, presented in the summary of data for dilution variance, this method reduces the effect of dilutions from that of the 1 : 10 for fungi shown above to the following:

	Fungi, 1 : 50 dilution		
P	1.00 – 0.20	0.20 – 0.05	0.05 – 0.00
Actual	25	6	3
Theoretical	27.2	5.1	1.7
A/T	.9191	1.1765	1.7647

Since there is such a close agreement in the ratios of actual to theoretical for fungi and bacteria when two dilution blanks were used for raising, it is probable that a similar improvement would result if one dilution blank only were used for raising to the dilution required for plating bacteria. This would mean the use of much larger dilution blanks than is the general practice.

The possibility of variation in dilution blanks introducing error in the technique was recognized early. Accordingly, over 400 blanks selected at random after sterilization were measured in a 100-ml. burette. They were found to vary as much as $\pm 3\%$. This is a greater tolerance than the $\pm 2\%$ allowed in the Standard Methods of Milk Analysis (1). The pipettes used were uniform in type, but were not checked for tolerance.

Another cause of the error of dilutions probably is the rapid settling of the soil in the 1 : 10 dilution before the first transfer is made. In other words, the mixture of soil in water does not represent a uniform suspension. The relatively better result for counts of fungi may be due to the size of the fungal spores and pieces of mycelium. These would be separated from the soil particles more readily and would remain suspended longer than soil particles, which carry the bacteria down.

The use of 1240 ml. of water to suspend 25 gm. of soil in the original dilution provides a marked improvement in technique. The variance among dilutions for counts of fungi is reduced to insignificance by this procedure. Accordingly, one dilution may suffice to provide an estimate of the population in the 25-gm. aliquot sample used. This is not so for counts of bacteria. The variance among dilutions, although reduced by this method of diluting, is still highly significant.

This being the case, there is no alternative other than to suggest the use of a 1 : 50 original dilution and the replication of dilutions, rather than the replication of plates from one final dilution. This is shown clearly in the data for systematic error. The variance for columns is not significant. This means that one plate from each of six dilutions produces an estimate that does not differ significantly from that of another plate from each of the same dilutions. The greater the number of replicate dilutions the more accurate the estimate of the population in the sample diluted. Again, it must be realized that the result at this stage provides merely an estimate *on the 25-gm. aliquot sample* used in preparing the dilutions.

The data show conclusively that one 25-gm. aliquot sample does not provide an accurate estimate of the population of fungi or bacteria in the sample brought to the laboratory. Further, the variation among aliquot samples is much greater than that among replicate dilutions of one aliquot sample. Accordingly, this furnishes the most serious error in the technique. It should be given first consideration and more weight than the error of dilutions or of replicate plates. As a suggestion, if one is to limit the study to 18 plates prepared from one laboratory sample, six aliquot samples should be plated in three replicate dilutions with one plate for each dilution. The data presented do not justify outlining a definite procedure in this respect, but substantiate the conviction that, until some improved method of reducing the error of the aliquot sample and that of the dilution has been established, little valid information can be obtained with less labour.

The interest in systematic error rests wholly on significance. The data show clearly that, compared to the error variance, the minor errors of the technique are not significant. This does not lessen the necessity for carrying out the procedure in a careful and orderly manner. At the same time, nothing practical may be expected to result from certain refinements in technique that may seem important in the plate method. In this study the first plate prepared, poured and counted did not differ significantly from any other in the set. The same was true of the bottom plate in the pile.

Probably the chief problem in obtaining an estimate of the population per gram of soil in a given field has not been considered in this presentation. It is obvious that an estimate of the population in a laboratory sample has little value if it cannot be related to the field soil. It is equally clear that, if the estimate on the laboratory sample is not accurate, valid information on the population cannot be obtained. On this assumption this important point has been left for later study.

Finally, the data presented appear to provide sound reason for the uncertainty about the value of the plate method referred to in a previous paper (3). Likewise, they suggest that much that has been done in an attempt to apply counts of micro-organisms to certain conditions or treatments may be worthless. Each of the above two statements is a condemnation of the method as it has been used. Neither has any relation to the method that may be developed by the application of statistical methods to prove the validity of each step in the procedure.

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THE AEROBIC DECOMPOSITION OF GLUCOSE IN PODSOL SOILS[1]

By P. H. H. Gray[2] and C. B. Taylor[3]

Abstract

The decomposition of glucose in samples from cultivated podsol soils of the Appalachian upland region of Quebec Province was effected rapidly by aerobic micro-organisms without the aid of added nitrogen. Potassium nitrate stimulated the rate of carbon dioxide production from glucose added to soil; the rate increased chiefly during the early stages while glucose was still present, and bacterial numbers were rising. Fungi developed high numbers later than the bacteria; they developed especially in soil to which glycine was added with the glucose. Biological activity was stimulated in soils in which glucose had previously been decomposed. The decomposition of the glucose appears to release other sources of available food material.

Introduction

Among the factors that have an essential bearing on the decomposition of carbonaceous residues in soil, the nature and amount of available nitrogen would appear to be of first importance. It has been found that the addition of available nitrogen, in the form of salts of ammonia or of nitric acid, or in manure, stimulates the decomposition of cellulose (2). It has also been shown that the organisms in soils that have not received such treatments are capable of decomposing cellulose, though at a much slower rate. This is usually accounted for by the existence in the soil of a small amount of some form of available nitrogen. The concentration of nitric nitrogen present in soil appears to have some effect on the amount of cellulose decomposed; in fact, the relation is masked when an excessive amount of nitrate nitrogen is added (15). The nature of the nitrogen compounds, other than ammonium salts, nitrites and nitrates, available to the organisms in soil is not known, but they are assumed to be adsorbed ammonium compounds derived from the decomposition of soil protein. Nitrate nitrogen would appear to be the most important source of nitrogen for the heterotrophic micro-organisms.

It has been established that the decomposition in soil of plant materials, such as straw, is controlled by the availability of the organic nitrogen in the material or, as it is generally stated, by the ratio of carbon to nitrogen. Material that has a high C : N ratio is decomposed more rapidly if ammonium or nitrate nitrogen is added to supply its natural deficiency in available nitrogen (1, 12, 14); that is, the C : N ratio must be lowered if microbial decomposition of the carbonaceous material is to proceed efficiently.

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Organic compounds containing no nitrogen, such as decomposition products of cellulose or cellulosic material, may be a cause of the temporary removal of nitrogen and result in deficient plant growth. This is generally accepted as the reason for lowered yields after the application of straw to soil, or its use as a mulch. Such treatments may, however, ultimately result in gains through the use of the carbonaceous material by nitrogen-fixing bacteria, whose cells will in time become part of the nitrifiable soil protein (8).

A high C : N ratio in soil would indicate that some conditions exist that prevent the micro-organisms from decomposing the carbonaceous residues efficiently. It has been suggested that heavily leached soils owe their high content of organic matter chiefly to climatic conditions in which the decomposition of plant residues proceeds slowly, and that leaching of the acidic products induces a continuation of low base-saturation after the soils have come into cultivation. The studies so far made of cultivated podsol soils of the Appalachian uplands of Quebec Province have shown that nitrogen alone, in the form of sodium nitrate, does not increase microbial activity. They have shown, also, that the addition of caustic amendments results in greater biological activity, not only in the total flora, but also in the formation of nitrates, thus indicating that nitrifiable material had been released from the organic complex; the freed nitrogen evidently enabled the heterotrophic organisms to decompose more of the carbonaceous residues in the soil (5).

Although soil organisms are able to decompose more cellulose in the presence of added nitrogen compounds than in their absence, considerable time is needed, and it is possible that some of the decomposition is due to other forms of nitrogen already present or developing in the soil. It is also probable that the decomposition is carried out by an association of organisms, and not by a restricted flora, the decomposition resulting in a number of products of different energy values; this may also be true in the decomposition of starch. It was thought, therefore, that it would be useful to study the course of the decomposition of substances that would encourage the rapid development of a restricted flora, in order to determine if the addition of nitrogen would stimulate the organisms during the utilization of material supplying readily available energy.

Under cultural conditions micro-organisms may produce glucose from cellulose (9), and maltose, with or without glucose, from starch. In view of the fact that glucose can be so easily decomposed by many micro-organisms, both aerobic and anaerobic, it seemed useful to investigate some of the factors that operate in the aerobic decomposition of glucose in soil samples under controlled conditions. The present study was made as an attempt to ascertain the effects of mineral and organic nitrogen compounds on the decomposition of glucose in samples of field soil of the type mentioned.

Experimental

Glucose is used in laboratory studies of microbial activities in soil, especially for obtaining enrichment cultures of nitrogen-fixing bacteria. When added

to soil under the appropriate conditions of oxygen tension, moisture and temperature, *Azotobacter* may develop in the presence of atmospheric oxygen, and *B. amylobacter* if the oxygen be reduced or removed. It had been previously ascertained that *Azotobacter* was absent from these soils, even after the addition of lime. The conditions of the experiments to be described were such that adequate aeration of the treated samples would have enabled an aerobic flora to develop, so that *B. amylobacter* or other anaerobic bacteria would not be encouraged. It was, otherwise, no part of the present studies to ascertain the cultural characters of the bacteria that utilized the glucose.

The soil samples were taken from field plots under experiment in or near Sawyerville, Compton County, in the Eastern Townships district of Quebec Province. The soils are derived from black and grey Ordovician slates, and have an organic carbon content of from 3 to 6%; the ratio of organic carbon to nitrogen is about 15 : 1 (4). Samples were collected by spade from the top 6 in., passed through a 2-mm. sieve, and allowed to dry rapidly in the air of the laboratory. After the glucose and nitrogen compounds had been added, the dry soils were moistened with distilled water to give 60% of the water-holding capacity, which was determined by the funnel method (10). The required amount of water was added slowly from a pipette; when the soil at the bottom of the dish was wet, the moist soil was thoroughly mixed.

The utilization of glucose, added as a 0.5% solution, was determined by the method of Bertrand (3). Biological activity was determined by estimation of the amount of carbon dioxide evolved during a period of about 10 days at room temperature (25 to 28° C.). The numbers of bacteria were estimated by plating with soil extract glucose agar, the same medium being used, after acidification with sterile tartaric acid solution, to count the fungi. Nitrates were determined by Harper's modified phenoldisulphonic acid method (7).

For the evolution of carbon dioxide the treated samples were placed in suction flasks of 1000-ml. capacity, and the gas was absorbed by means of barium hydroxide, as described previously (10). Samples for the determination of sugar, for numbers of bacteria and fungi, and for nitrates, were placed in large culture dishes, to permit adequate penetration of atmospheric oxygen.

The following experiments were made:

(i) A preliminary experiment to ascertain the rate of decomposition of glucose in the presence and absence of nitrate nitrogen.

(ii) An experiment to determine the effects of nitrate in single and double amounts.

(iii) An experiment to determine the effects of soil nitrates (developed in samples incubated for 30 and 56 days) and the effects of a previous treatment with glucose.

(iv) An experiment to determine the effects of the amino-acid glycine.

In each experiment a sample of soil having only water added to it was used as a control.

EXPERIMENT 1

The samples used in this experiment had been dried, before nitrate nitrogen could accumulate, and stored in the laboratory. A test showed that there was no nitrate nitrogen present in the soil. The treatments were (i) glucose alone, (ii) glucose plus potassium nitrate, and (iii) untreated control. The amount of potassium nitrate added was sufficient to give 16.7 p.p.m. of nitrate nitrogen in the moistened soil. The amount of glucose added was equivalent to 30.9 mg. per 10 gm. of moist soil.

The results of the determinations of glucose, of nitrate nitrogen, and of the estimations of bacterial numbers are given in Table I.

TABLE I

Time, hr.	Glucose in 10 gm. moist soil, mg.		Nitrate nitrogen, p.p.m.	Bacteria, millions per gm.		
	Glucose	Glucose $+KNO_3$	Glucose $+KNO_3$	Glucose	Glucose $+KNO_3$	Control
At start	29.0	28.0	16.9	0.9	0.9	0.9
12	28.0	28.0	13.8	1.0	1.0	1.1
24	27.0	26.0	15.9	2.3	3.1	6.5
48	15.4	9.5	Nil	31.1	40.1	15.8
72	4.4	1.1	—	35.2	33.8	18.2
96	Nil	1.1	—	42.0	40.9	15.6
120	Nil	Nil	—	40.7	42.0	23.5

The disappearance of the added nitrate, between the 24th and 48th hr., was associated with the sudden decrease in the amount of glucose and with the rise in bacterial numbers. The nitrate does not appear to have effected any difference in numbers; the difference observed between the amounts of glucose remaining at the 48th hr. may not be significant. The maintenance of high numbers after the sugar had been utilized is commonly found after treatments that stimulate a restricted flora.

The carbon dioxide production was determined from duplicate 100-gm. portions of soil; the total amounts of gas evolved from the cultures in 12 days are given below.

TABLE II

EVOLUTION OF CARBON DIOXIDE, MG. PER 100 GM. OF SOIL

Sub-sample	Glucose	Glucose $+KNO_3$	Control
a	571	584	260
b	553	572	254
Mean	562	578	257
Minus control	305	321	–

The nitrate appears to have increased activity. The effect can be seen by determining the percentage difference between the mean of the two cultures receiving glucose, after subtracting the mean values of the controls. The mean difference due to nitrate was only 5%, which, in view of the differences found between parallel sub-samples, cannot be considered significant.

Since, however, the effects of the nitrate may have occurred only during the time when the glucose was being utilized and while the bacteria were increasing in numbers, it seemed probable that the values for the amounts of carbon dioxide produced during that time would show greater differences. The amounts of carbon dioxide were therefore determined for each interval in the manner described below.

It was the practice in this and later experiments to titrate the barium hydroxide in the first two absorption tubes at intervals as seemed necessary, before all of the barium hydroxide had been changed to the carbonate; that in the third tube was titrated at the end of the experiment. In order to show the rate of evolution, values were derived by determining the proportion that the amounts found in the first tube at each interval bore to the total amount and by calculating the amounts in the second and third tubes as proportionate for each interval. The values given in Table III represent the calculated amounts in tubes 2 and 3 added to the known amounts in tube 1. The values may be accepted as true if it may be assumed that the amounts of carbon dioxide passing over from tube 1 to tube 2, and from tube 2 to tube 3, were regularly proportionate throughout the experiment. From the observed values, the amounts of carbon dioxide evolved per hour were calculated; these values are also shown in Table III.

The latter values bring out the fact that the increase due to nitrate was considerably higher during the earlier stages, namely, between the 32nd and the 50th hr. The period of greater effect corresponds to that during which the bacteria were increasing at the greatest rate, and that during which, as shown in Table I, the glucose was most rapidly decomposed.

TABLE III

EVOLUTION OF CARBON DIOXIDE FROM 100-GM. SAMPLES OF SOIL

Time, hr.	Total amount, mg.			Rate, mg. per hr.		
	Glucose	Glucose +KNO_3	Control	Glucose	Glucose +KNO_3	Control
25.50	61.85	54.37	40.42	2.43	2.13	1.59
32.25	48.65	52.45	–	7.21	7.77	–
41.75	54.32	76.06	28.45	5.73	8.02	1.75
49.25	37.58	47.08	–	5.01	6.28	–
65.75	63.40	66.35	–	3.84	4.02	–
80.25	47.74	41.37	45.70	3.29	2.85	1.19
120.00	75.64	74.25	30.53	1.89	1.86	0.76
168.00	66.06	64.04	38.25	1.38	1.34	0.80
288.00	107.04	102.33	73.54	0.89	0.84	0.60

EXPERIMENT 2

Since 16.7 p.p.m. of nitrate nitrogen stimulated the evolution of carbon dioxide, an experiment was made to test the effect of double that treatment. In this experiment, four 600-gm. samples of fresh air-dried soil containing no nitrate nitrogen were treated as follows: (i) 3.0 gm. glucose; (ii) 3.0 gm. glucose, 0.195 gm. potassium nitrate; (iii) 3.0 gm. glucose, 0.390 gm. potassium nitrate; (iv) no treatment. A 100-gm. portion of the moist soil of each treatment was used for determination of carbon dioxide production; the remainder was incubated in the dish for determination of glucose and nitrate, and the numbers of bacteria and fungi.

The amounts of glucose found in the samples are shown in Table IV.

TABLE IV

GLUCOSE, MG. PER 10 GM. OF SOIL

	Glucose	Glucose $+KNO_3$	Glucose $+KNO_3 \times 2$
Glucose added	30.8	30.8	30.8
Found, at start	28.4	28.4	28.4
Day 1	19.0	20.3	19.6
Day 2	6.0	5.0	7.5
Day 3	Nil	Nil	Nil

The glucose disappeared at the same rate in the three cultures.

The amounts of carbon dioxide collected in 276 hr. were as shown in Table V.

TABLE V

EVOLUTION OF CARBON DIOXIDE FROM TREATED SOIL

	Glucose	Glucose $+ KNO_3$	Glucose $+ KNO_3 \times 2$	Control
	Milligrams per 100 gm. of soil			
From cultures	373	405	406	95
Minus control	278	310	311	—
Time, hr.	Milligrams per 100 gm. per hr.			
22.0	3.12	2.70	2.49	—
36.0	4.94	6.04	5.14	—
51.0	3.90	4.60	5.23	—
75.5	2.32	2.30	3.10	0.52
123.0	0.82	0.95	1.02	—
192.0	0.69	0.75	0.70	0.31
276.0	0.42	0.52	0.32	0.23

The effect of nitrate nitrogen, estimated after subtracting the value of the control culture, was to increase the total carbon dioxide by 11.5%.

The amounts of carbon dioxide collected at intervals were also calculated, in the manner described, in order to arrive at the effects of the treatments on the rates of evolution; the results are given in Table V. The nitrate appears to have depressed the evolution of carbon dioxide at the start, but to have increased it after the 24th hr. The double amount was the more effective between the 3rd and 5th days.

The amounts of nitrate found in the cultures to which it was added, and the numbers of bacteria in all cultures, are given in Table VI. The nitrate was apparently being utilized at about the same rate in both cultures to which it was added, until the end of the 3rd day, after which 53 to 55% of the nitrate in the culture with the double amount remained until the end of the experiment.

TABLE VI

Time, days	Glucose	Glucose + KNO_3	Glucose + $KNO_3 \times 2$	Control
	Nitrate nitrogen, p.p.m.			
At start	—	33.4	60.0	Trace
1	—	—	—	Trace
2	—	13.2	47.0	Trace
3	—	Trace	27.1	—
5	—	—	27.1	—
12	Nil	Nil	28.5	18.9
	Bacteria, millions per gm.			
At start	5.2	—	—	—
1	63.9*	44.8	36.8	10.6*
2	154.5	203.0*	252.8	4.9
3	97.5	192.0	151.6*	5.6
4	120.1	145.0	135.2	6.8
5	46.9	46.1	32.8	7.6
	Fungi, thousands per gm.			
7	143	196	220	118

* χ^2 *excessive.*

The increases in the amounts of carbon dioxide, in the cultures receiving nitrate, may be ascribed to the higher numbers of bacteria that developed during the period synchronizing with the greater rate of evolution of the gas. The nitrate appears also to have depressed the increase in bacterial numbers at the 24th hr. (See also Fig 1.)

The numbers of fungi were estimated on the 7th day in plates of soil extract glucose agar rendered acid with tartaric acid. While not conclusive evidence of the course of development of these organisms, the numbers found are of interest, and are given in Table VI.

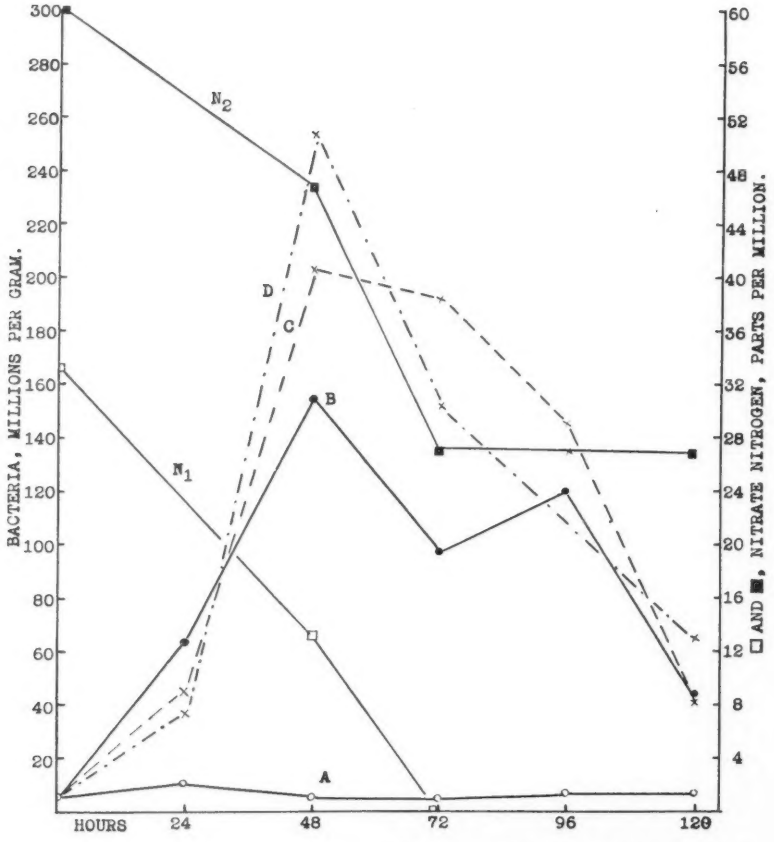

FIG. 1. *Numbers of bacteria in:— A, control; B, glucose; C, glucose + potassium nitrate; D, glucose + potassium nitrate (double amount); and nitrate nitrogen in C (N_1) and D (N_2).*

As shown in a later experiment, the fungi developed slowly, and reached their maximum numbers after the glucose had been utilized, during the period that bacterial numbers were decreasing. This would suggest that some source of organic carbon, and with it some organic nitrogen, or ammonia, were released during that period.

It would appear that the complete utilization of the sugar at about the end of the 3rd day set a limit to the further use of about half of the nitrate in the sample receiving the double amount. The fact that bacterial numbers fell rapidly with the disappearance of the glucose and remained stationary between the 5th and the 11th days suggested that there was no further source of energy remaining for these aerobic bacteria; that is, the glucose was completely

oxidized. The carbon dioxide found, therefore, after the 3rd day must have been due to respiration of diminished numbers of bacteria, coupled with that of apparently increasing numbers of fungi, developing from some source of energy released through bacterial action and from a source of nitrogen other than nitrate nitrogen.

The maximum rate of evolution of carbon dioxide occurred at about the 36th hr., which corresponds to the time when half of the energy material had been utilized (Table V). In view of the fact that the rate of evolution at the 75th hr. was, on the average, about half the rate at the 36th hr., it is probable that the whole of the glucose was utilized at about the 72nd hr., when the final determination was made for glucose. This seems to be a reasonable assumption, as half the glucose had gone at the 36th hr. About half of the nitrate nitrogen in the culture that received the single amount had also been utilized at that time.

It may be useful to submit a calculation regarding the relation of carbon dioxide produced to the theoretical amount possible at the 72nd hr. The amount of gas evolved from the sugar at that time may be taken as 220 mg., which represents about 41% of the theoretical maximum amount. The total amount of gas produced from glucose in the culture with added glucose only was 373 − 95 mg. = 278 mg.; this represents 66.7% of the theoretical maximum amount. The average amount evolved in 11.5 days from the three cultures receiving glucose, 300 mg., represents 72%. Merrill (11) states that in pure cultures of *Mycobacterium*, a group of aerobic bacteria that oxidize glucose, 85% of the theoretical maximum amount may be obtained. In soil cultures there is the probability to be considered that products other than carbon dioxide may be formed and utilized.

If the rates of evolution at 276 hr. be compared, it is clear that some time would elapse before the rates from the cultures with glucose would fall to the value given by the control soil.

Tests by the colorimetric method with samples that had an initial pH of 5.06 showed that after glucose had been decomposed the pH had been raised to 7.0; distillates from the samples were also approximately neutral.

It is clear that there was sufficient nitrogen available in the soil to allow the bacteria in the sample receiving glucose alone to utilize all of the available sugar in the same time as in the samples receiving nitrate.

Experiment 3

The samples of soil used in the previous experiments contained no nitrate nitrogen other than that supplied as potassium nitrate. In this experiment, samples of fresh soil from the same source were incubated for a sufficient length of time to allow the nitrifying bacteria to develop a considerable content of nitrate through the oxidation of the ammonium, or amino-, compounds of the soil protein. The effect of the removal, or at least of the utilization, of available nitrogen by previous decomposition of glucose was also tested.

After the soils had been incubated in the moist state, for different lengths of time, they were spread out to dry rapidly. The treatments are summarized in Table VII. Nitrates were then determined, and plates prepared for counting bacteria and fungi; glucose was then added and the samples were moistened. It was expected that some of the effects might be due to the length of time the samples had remained in the air-dry state; therefore, a sample of each series without glucose was also moistened and incubated, to serve as a control. The results are given in Tables VII, VIII, and IX.

As shown in Table VII, the glucose disappeared rapidly, all of it being utilized within 48 hr. The nitrate nitrogen does not appear to have stimulated the decomposition.

TABLE VII

TREATMENT OF SAMPLES, NITRATE CONTENT, AND UTILIZATION OF GLUCOSE

Series	Incubation previous to air-drying and moistening, days	Time air-dried, days	Nitrate N after air-drying, p.p.m.	Glucose added per 10 gm. soil, mg.	Glucose found, mg.		
					At start	Day 1	Day 2
I	Nil	71	Trace	30.8	30.5	17.9	Nil
II	30	41	32–33	30.8	30.5	18.2	Nil
III	56	15	41–42	30.8	30.5	19.4	Nil
IV	30 with glucose	41	Trace	30.8	30.5	12.0	Nil

The evolution of carbon dioxide in 10 days and the amounts produced per hour are shown in Table VIII.

TABLE VIII

EVOLUTION OF CARBON DIOXIDE FROM 100-GM. SAMPLES OF SOIL

Series		I	II	III	IV
		Total amount in 10 days, mg.			
Cultures with glucose		418	404	424	468
Control cultures		120	94	77	178
Minus controls		298	310	347	290
	Time, hr.	Rate, mg. per hr. (from first 2 baryta tubes)			
Cultures with glucose	24	3.20	3.22	2.62	4.07
	36	7.05	7.59	8.89	7.75
	55	4.56	4.92	5.78	4.48
	144	0.86	0.85	0.86	0.86
Control	55	0.93	0.72	0.61	1.37

In a discussion of the results, it is first necessary to consider the effects of drying for the different periods. The effect can be seen best in the control cultures of Series I, II, and III, which had remained in the air-dry state for 71, 41, and 15 days respectively. The longer the period of drying, the greater was the amount of gas evolved. The effects were masked by the decomposition of glucose to such an extent that, in considering the total amount of gas produced from the cultures, no real differences can be seen. The amounts of carbon dioxide derived from the glucose may, therefore, be ascribed to other factors affecting the micro-flora, which by the treatment with glucose became restricted to one or two types of micro-organisms. The amount of gas produced from glucose in Series II, which contained 32–33 p.p.m. of nitrate nitrogen, was about 4% more than that from Series I; that from Series III, about 16%. In view of the close agreement obtained between duplicate cultures in a later experiment, reported below, it is possible that both of those differences are significant.

The effects of the previous treatment with glucose can be seen both in the control cultures and in the cultures receiving the second addition of glucose; the comparison should be made only between the cultures of Series II and IV, which had remained dry for the same number of days. Previous decomposition of glucose did not increase the amount of carbon dioxide produced from the second addition, but reduced it by about 7%, which may be significant. The previous treatment did, however, stimulate a more rapid initial evolution after the second addition, the increased rate, calculated for the 24th hr., being 57% higher. The greater effect of the previous treatment is seen in the control cultures, in which the increase in the total amount of gas was 89%.

It would appear, then, that the previous development of organisms set up some condition that did not stimulate the utilization of another supply of the same energy material, except in the first few hours. The decomposition of glucose in this soil seems, therefore, to be of a nature different from that taking

TABLE IX

NUMBERS OF BACTERIA AND FUNGI, INCUBATION EXPERIMENT

Series		I	II	III	IV	I	II	III	IV
	Time, days	Bacteria, millions per gm.				Fungi, thousands per gm.			
Cultures with glucose	At start	1.3	2.4	3.3	8.4	98	74	90	132
	1	19.7	19.8	8.1	25.5	28	7	53	53
	2	29.3	47.1	57.1	62.1	62	100	72	155
	3	63.3	59.3	53.6	87.1	82	217	290	286
	4	37.4	73.5	86.2	76.0	110	342	457	412
	6	47.3	61.1	69.4	78.4	162	507	610	592
Control cultures	At start	1.3	2.4	3.3	8.4	98	74	90	132
	2	4.4	5.6	5.1	2.2	90	104	86	119
	4	14.4	9.3	9.7	34.7	170	115	147	167

place when successive additions of a hydrocarbon are decomposed. Sen Gupta (13) showed that second and third additions of phenol were decomposed at increasingly rapid rates in Rothamsted soil.

The numbers of bacteria and fungi are shown in Table IX. From the results shown for bacterial numbers, it would appear that the nitrate formed in the incubated soil had no influence on the development of these organisms. The increased numbers of fungi in Series II and III suggest that they were stimulated by the nitrates, but the numbers in Series IV would rule out that suggestion. The increases in the numbers of the fungi would seem to confirm the view expressed above, namely, that bacterial activity may have released some form of food material, containing both carbon and nitrogen, for these organisms. The higher numbers in the control culture of Series IV at the start gives emphasis to this view.

Experiment 4

This experiment was made to ascertain if an amino-acid would serve as a source of carbon and nitrogen, and if it would stimulate the decomposition of glucose.

The samples were derived from the same source as those used in the two previous experiments. There was only a trace of nitrate nitrogen present.

The following cultures were made: (i) 400 gm. of soil, with 2.0 gm. of glucose; (ii) 400 gm. of soil, with 0.112 gm. of glycine; (iii) 400 gm. of soil, with glucose and glycine; (iv) no treatment.

The glycine supplied 52 p.p.m. of nitrogen. Duplicate 100-gm. portions of soil were used for the evolution of carbon dioxide; the remainder was used for the determination of glucose, and for the estimation of numbers of bacteria and fungi. The results are shown in Tables X, XI, and XII.

The glucose was used up rapidly, none being left in the soil on the second day.

The amounts of carbon dioxide collected in 10 days were as shown in Table X.

TABLE X

Carbon dioxide, mg. per 100 gm. of soil

	a	b
Soil + glucose	401	401
Soil + glycine	153	157
Soil + glucose + glycine	461	464
Control	113	113

The amino-acid evidently stimulated biological activity to an appreciable extent: the increase of the glycine cultures over the control cultures was 37%; the increase caused by glycine in the cultures receiving both chemicals over those receiving glucose only, was 15%. In view of the close agreement between duplicate cultures, this is probably a real difference. The differences among

the effects of the treatments are more clearly brought out by comparing the rates of evolution, given in Table XI.

TABLE XI

CARBON DIOXIDE, MG. PER 100 GM. PER HOUR

Time, hr.	Cultures receiving glucose			
	Glucose		Glucose + glycine	
	a	b	a	b
28.5	3.02	2.99	2.92	2.92
36.5	8.60	9.31	10.27	10.55
46.0	5.58	5.38	9.37	9.66
60.5	4.37	4.40	4.03	4.87
142.0	1.05	1.04	1.12	1.11
238.5	0.46	0.44	0.44	0.45
	Cultures without glucose			
	Glycine		Control	
	a	b	a	b
70	1.26	1.35	0.92	0.92
238.5	0.39	0.37	0.29	0.29

Although glycine alone stimulated activity to such an extent that the rate of production was increased 41% at the end of the 70th hr., the greater effect of the glycine occurred in association with glucose; at the 46th hr. the increase due to glycine was 77% (see also Fig. 2).

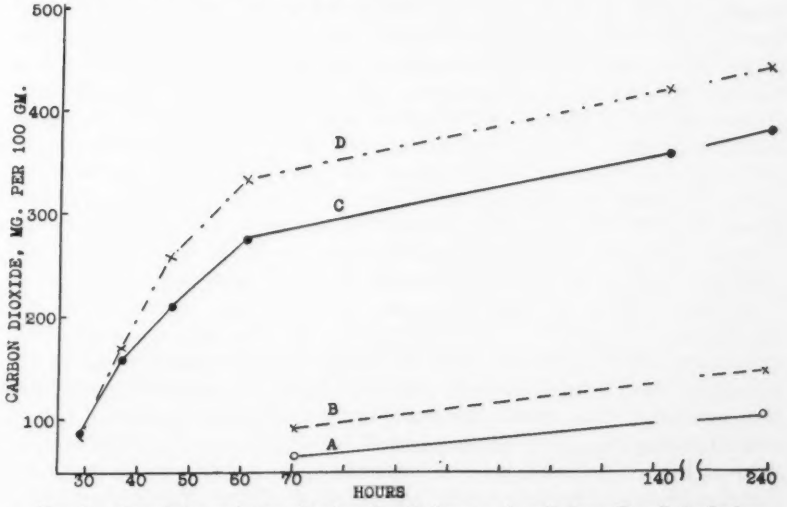

FIG. 2. *Cumulative evolution of carbon dioxide from:— A, soil alone; B, soil + glycine; C, soil + glucose; and D, soil + glucose + glycine.*

TABLE XII

NUMBERS OF MICRO-ORGANISMS, GLYCINE EXPERIMENT

Time, days	Glucose	Glucose + glycine	Glycine	Control
	Bacteria, millions per gm.			
At start	1.9	1.9	1.9	1.9
1	16.2	4.3	2.7	5.8
2	84.7	45.4	5.0*	9.2
3	66.9	73.2	14.1	8.3
4	74.4	83.1	26.1	10.5
7	75.4	71.7	37.5	16.5
	Fungi, thousands per gm.			
At start	101	101	101	101
1	197	212	41	107
2	110	232	100*	86
3	282	800	220	112
4	287	1060	195	102
7	570	960	267	77

* *Approximately; too many colonies in the plates for an accurate count.*

Bacterial numbers were depressed by the glycine until after the 48th hr., when they began to rise and were nearly double the numbers in the control cultures at the 4th and 7th days.

Reference to Table XII shows also that the numbers of bacteria in the samples receiving glucose were not increased by the glycine, but were depressed until the 4th day. The increase in the carbon dioxide could not therefore be ascribed to the bacteria alone, since at the time of the greatest increase in rate, at the 46th hr., the numbers in the culture with added glucose and glycine were about equal to only half the numbers that developed in the sample with glucose alone. The fungi, on the other hand, were greatly stimulated by the glycine added with the glucose; the greater effect was not evident until the glucose had disappeared. The increase in numbers of fungi caused by the glycine was relatively less than that of the bacteria; by taking the mean numbers of the two cultures with glucose, it will be seen that the fungi multiplied from 7 to 8 times in 7 days, while the bacteria increased about 35 times in 3 days. Most of the carbon dioxide must have been produced by the bacteria, whose numbers did not become less while the fungi were increasing. Numbers alone, however, are not an adequate basis of comparison between these two kinds of micro-organisms, as they differ not only in size, but also in their mode of using sources of energy and nutrition.

The decomposition of glucose evidently released some source of food that enabled the fungi to develop to a considerable extent after the bacteria had multiplied; this was confirmed in another experiment. The glycine, in the presence of glucose, was apparently utilized by the fungi at the time that the

bacteria were also increasing in numbers, but the competition of the increasing fungi caused the bacteria to multiply at a slower rate than that produced by glucose alone.

The results of this experiment suggest that the natural flora had been displaced not only by a selective group of bacteria that obtain their energy from the added carbohydrate, which enables the cells to utilize some source of organic nitrogen, but also by a group of fungi that develop by utilizing decomposition products of the glucose or material released from the organic matter during and following the destruction of the carbohydrate. It would also appear that the fungi, developing somewhat more slowly than the bacteria, preferred nitrogen derived from the added amino-acid rather than the nitrogen in the soil. Reference to the results of the counts for fungi in Experiments 3 and 4 would seem to confirm this, and to suggest that nitrates, either added in the form of potassium nitrate or developed through nitrification, are less suitable sources of nitrogen than glycine for the fungi in soil.

Conclusions

The results from these experiments with samples of field soil would seem to confirm the view expressed in a previous paper dealing with virgin soils (6), namely, that water-soluble materials in soil include substances that are able to support the growth of micro-organisms.

In regard to the cultivated soils studied in the present work, the relation between the various factors in the complex material that supplies energy or food for the micro-flora is somewhat different from that in virgin soils of this type; the organic carbonaceous residues are in a more advanced stage of decomposition, as is shown by the higher levels of numbers of organisms and by the lower yield of carbon dioxide (6) in the cultivated soils. This would suggest a reason for the fact that nitrate nitrogen alone does not stimulate further development of the micro-flora. It would appear from the results reported above that the nitrogenous material remains in a state of availability, or quickly becomes available, because additional sources of easily available carbon are rapidly destroyed by an increasing number of bacteria. The development of filamentous fungi in the soils after the decomposition of the carbonaceous material, and after the bacterial numbers have reached their peak, suggests that food material containing both carbon and nitrogen had been released.

The experiments reported here can be regarded only as part of an attempt to determine the nitrogen metabolism of aerobic soil micro-organisms, which probably, under normal cultural practices, are responsible for the decomposition of soluble carbonaceous compounds in the organic residues in soil. The method adopted was designed to stimulate only those groups of organisms able to utilize a single source of additional energy, within a period of time in which it would be possible to evaluate their action without the interference of competitive groups, such as may develop when a more complex material,

for example cellulose, is added to soil. It seems clear that although nitrate nitrogen stimulated the evolution of carbon dioxide during the period of maximum development of bacteria, the additional nitrogen did not enable the organisms to utilize the glucose more rapidly than they did in soil to which nitrate nitrogen was not added.

Acknowledgment

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LAND AND FRESHWATER MOLLUSCA FROM WESTERN ONTARIO[1]

BY FRANK COLLINS BAKER[2]

In the summer of 1931, Dr. A. R. Cahn, then assistant professor of Zoology in the University of Illinois, made a canoe trip over the lakes and rivers of the International Boundary between Canada and the United States and the waters immediately to the north and east of this boundary. In July, 1935, Dr. Cahn made a similar trip through a portion of the Lake of the Woods region. Dr. R. G. Lindenborg, a graduate student in the Department of Zoology, made collections in the Quetico Provincial Park, Rainy River District. The latter collection consisted principally of land molluscs while that of Dr. Cahn was made up largely of freshwater species. The present paper may be considered supplementary to that of Baker and Cahn (6) on freshwater Mollusca from central Ontario. Some of the data on Planorbidae were published by Baker (4).

In the paper by Baker and Cahn, the material listed was collected in Thunder Bay district and Rainy River district. In the present paper the itinerary was largely confined to the Rainy River district and included the following lakes: Crooked Pine, Pickerel, Sturgeon, Basswood, Agnes, Louisa, Knife, Kahnipiminanikok, Saganagons, and Saganaga. Many smaller lakes and connecting waters were also visited.

The 1935 trip made by Dr. Cahn was in the Lake of the Woods region, Kenora district. In outline the itinerary was as follows: Lakes Kakagi, Cedartree, Flint, Cameron, Otterskin, Hillside, Horseshoe, Pipestone, Kishkutena, Sabaskong Bay, Miles Bay, Tug Channel, and Whitefish Bay. A portion of the trip was around the Aulneau Peninsula, on the east side of Lake of the Woods.

The vast area of Lake of the Woods, with the myriads of small islands in the lake and the hundreds of lakes in the immediate region, is comparatively little known conchologically. Dawson's paper (7) is the most extensive report on the fauna of this region and lists 24 species, 14 gastropods and 10 pelecypods. The Cahn collection contained 16 gastropods and 5 pelecypods. Many small clams of the Sphaeriidae were not contained in the Cahn collection and some small gastropods listed by Dawson were also missing. Lake of the Woods is notable as being one of the type localities of Say's *Planorbis corpu-*

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lentus, one of the finest species of the genus *Helisoma*. The variety *multicostatum* also occurs in the lake (4).

A noteworthy contribution from the Cahn collection is the presence of *Helisoma campanulatum collinsi*, a new race of *campanulatum* abundant in the Lake of the Woods. A careful study of the Lake of the Woods region by a trained conchologist with abundance of time would doubtless increase the molluscan fauna to more than 50 species and races.

The present paper lists 22 species and races not included by Baker and Cahn (6).

The writer is greatly indebted to both Dr. Cahn and Dr. Lindenborg for the collections made in Ontario, which have been given to the University of Illinois and form a part of the collection of Mollusca of the Museum of Natural History. Thanks are due to Dr. W. J. Clench, of the Museum of Comparative Zoology, for the identifications of certain *Physa*, and to Dr. Harold A. Rehder, of the U.S. National Museum, for the identification of certain *Succinea*.

Pelecypoda

Family Unionidae

Anodonta grandis footiana Lea

Rainy River district: Baird Lake; Keats Lake; Athelstane Lake; Crooked Pine Lake; Basswood Lake; Kahnipiminanikok Lake; between Saganagons and Othermans Lake; rapid water between Saganagons and Kahnipiminanikok Lakes; Greenwood Lake; Camel Lake; Ogish-ke-muncie Lake; Mowe Lake; Silver Lake; Lasper Lake; Sark Lake; Fern Lake; Brent Lake. Thunder Bay district: Chief Peter Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; McPherson Island, near Sabaskong Bay; Astron Bay, north side Aulneau Peninsula; Turtle Portage, north of Sabaskong Bay; Horseshoe Lake, east of Kakagi Lake; southeast side Sabaskong Bay, on small island; Sammy Lake, east of Kakagi Lake.

This *Anodonta* is the most abundant form of the genus in western Ontario. It is more elongated and less high than the specimens from the type locality in Winnebago Lake, Wisconsin, but is well within the range of variation of the race (1, p. 157). An average adult specimen measures well over 100 mm. in length and 50 mm. in height. The colour is dark yellowish-brown, sometimes with a decided greenish tinge. The later Cahn records widely extend the distribution of the race in Ontario.

Anodonta grandis plana Lea

Thunder Bay district: Waikwabauona River, flowing into Northern Light Lake. Rainy River district: river between Crooked Pine and Chief Peter Lakes; Pickerel River.

A large *Anodonta*, common to abundant in the first two localities cited above, appears referable to the creek form of *grandis*. It is large, attaining a length of over 100 mm., the shell is elongated with pointed posterior end. This race was not observed in the material listed by Baker and Cahn (6, p. 44).

Anodonta kennicottii Lea

Rainy River district: Basswood River rapids; McKenzie Lake.

A. kennicottii does not appear to be as abundant in Ontario as it is in Minnesota and Wisconsin.

Anodonta marginata Say

Rainy River district: Kekequebic Lake; Ogish-ke-muncie Lake; Crooked Pine Lake; Greenwood Lake. Thunder Bay district: Northern Light Lake. Kenora district (Lake of the Woods): Turtle Portage, north of Sabaskong Lake.

A. marginata appears more widely distributed in Ontario than was indicated by the collections previously made. Its greenish, fragile shell can scarcely be mistaken for any other species.

Anodontoides ferussacianus (Lea)

Rainy River district: Kashaboiwe Lake. Kenora district (Lake of the Woods): McPherson Island, near Sabaskong Bay.

A. ferussacianus was not observed in the material previously collected by Dr. Cahn in Ontario. Only two specimens were found in the material now under observation, and the species is probably to be considered as rare in this part of Ontario.

Lasmigona compressa (Lea)

Rainy River district: Kashahpiwigamak Lake; Kabwawiagamak River; river between Crooked Pine Lake and Chief Peter Lake.

L. compressa was not contained in the collections previously made by Dr. Cahn. The three localities listed above indicate that it occurs in western Ontario although, apparently, not abundantly, since in two of the localities only broken and partly worn shells were obtained. The specimens from the river between Crooked and Chief Peter Lakes were living and quite characteristic of the species. The largest specimen measures 87 mm. in length. The colour is dark olive with indistinct rays. Simpson (Cat. p. 482) gives the range as north to Wisconsin, but the Canadian records indicate a much more northern range than this.

Lampsilis superiorensis (Marsh)

Thunder Bay district: Obadinaw River. Rainy River district: Kabwawiagamak River; Kahnipiminanikok Lake; McKenzie Lake; Kashahpiwigamak Lake; Crooked Pine Lake; river between Crooked Pine and Chief Peter Lakes. Kenora district (Lake of the Woods): southeast side Sabaskong Lake, on small island; Rabbit Point, west of Sabaskong Bay.

Although bearing a strong resemblance to some forms of *L. siliquoidea* (Barnes), *L. superiorensis* appears to be sufficiently distinct and appears to be the prevailing *Lampsilis* in this part of Ontario, as remarked in a previous paper.

Lampsilis ventricosa canadensis (Lea)

Rainy River district: Fern Lake.

One female specimen of this race is contained in the Cahn collection. It is 78 mm. in length and 49 mm. in height and is greenish yellow in colour. This race should be found more abundantly than the collections would indicate.

FAMILY SPHAERIIDAE

Sphaerium sulcatum (Lamarck)

Rainy River district: Kashahpiwigamak River; between Saganagons Lake and Othermans Lake; Camel Lake; Mack Lake; lake west of West Lake and north of South Lake.

S. sulcatum was not abundant in the collections from Ontario previously listed (6, p. 46). It was common in several of the localities listed above.

Sphaerium fallax Sterki

Rainy River district: Greenwood Lake; Crooked Pine Lake; Kashaboiwe Lake. Thunder Bay district: South Arm, Saganaga Lake; Waikwabauona River, near Northern Light Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay.

S. fallax appears to be an abundant species of this genus in Ontario. It is variable but apparently quite distinct from its nearest relative, *sulcatum*.

Sphaerium striatinum Lamarck

Thunder Bay district: Northern Light Lake and Waikwabauona River, near Northern Light Lake.

A *Sphaerium* which appears to be this species is very abundant in the localities listed.

Sphaerium rhomboideum (Say)

Rainy River district: between Saganagons and Othermans Lakes, one typical specimen.

Apparently this *Sphaerium* is rare in Ontario.

Musculium securis (Prime)

Thunder Bay district: Waikwabauona River.

Abundantly represented in the collection.

Pisidium, species indet.

Rainy River district: Otter Track Lake.

A very small species of this perplexing genus. Since the death of Dr. Victor Sterki no one has taken up the study of these small bivalves, and the writer does not feel competent to attempt the identification of this species.

Gastropoda

Family Valvatidae

Valvata tricarinata (Say)

Kenora district (Lake of the Woods): Kennedy Island. Rainy River district: Sturgeon Lake.

The typically tricarinate form of the species.

Valvata sincera nylanderi Dall

Kenora district (Lake of the Woods): Kennedy Island.

Several characteristic specimens occurred in this collection. This is apparently the first record of *V. nylanderi* from this part of Ontario.

Valvata lewisi ontariensis F. C. Baker

The specimens listed as a discoidal form of *V. lewisi helicoidea* (6, p. 49) have been found to constitute a distinct race of *lewisi*. The types of this race are from Shakespeare Lake, near Nipigon Lake, Ontario, where the race occurs in some abundance (Nautilus, 44 : 119. 1931). The Cahn specimens came from Kimmewin Lake, near Drayton.

Family Viviparidae

Campeloma, species indet.

Rainy River district: Basswood River. Thunder Bay district: Northern Light Lake, east of Saganaga Lake. Kenora district (Lake of the Woods): McPherson Island.

Baker and Cahn (6) identified a *Campeloma* from Hamilton Lake as *milesii* (Lea). Until the whole genus has been carefully examined anatomically it is unsafe to identify positively these northern forms. Some specimens approach *milesii* in form and others might be referred to *decisum*. This genus is probably abundantly represented in the northern lakes but careful search is required to find them.

Family Amnicolidae

Amnicola limosa superiorensis F. C. Baker

Rainy River district: Kahnipiminanikok Lake; Sturgeon Lake; Kashaboiwe Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Kennedy Island.

The *Amnicola* referred to *porata* by Baker and Cahn (6, p. 49) is *superiorensis*, which is the common *Amnicola* in the lakes of western Ontario. The *Amnicola* listed as *porata*, from Prince Albert National Park, Saskatchewan, is also referable to *superiorensis* (5, p. 115). Typical *porata* must conform to the shells from the original locality, Cayuga Lake, N.Y. See Baker (1, p. 101) for definition of *superiorensis*.

FAMILY LYMNAEIDAE

Lymnaea stagnalis jugularis Say

Rainy River district: Basswood Lake; Snodgrass Lake; Kashaboiwe Lake; Emerald Lake; Carp Lake; Knife Lake; Mercutio Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Thunder Bay district: Lac des Mille Lacs swamp. Kenora district (Lake of the Woods): Otterskin Lake.

This typical form of the American *L. stagnalis* is apparently common in the lakes of Ontario.

Lymnaea stagnalis lillianae F. C. Baker

Thunder Bay district: Lac des Mille Lacs; Lac des Mille Lacs swamp.

This race appears to be common in certain lakes of Ontario. See the paper by Baker and Cahn (6) for additional records in Ontario.

Lymnaea stagnalis sanctaemariae Walker

Thunder Bay district: Lac des Mille Lacs swamp; Chief Peter Lake. Rainy River district: Crooked Pine Lake; Athelstane Lake; Emerald Lake; between Saganagons and Othermans Lakes.

The specimens referred to the race *sanctaemariae* have a somewhat longer spire than specimens from St. Mary's River in Michigan, but they all have the peculiar aperture of the race. The Ontario specimens vary toward *lillianae* on one hand and *jugularis* on the other. There is usually little difficulty in separating the three races of this polymorphic species.

Stagnicola lanceata (Gould)

Thunder Bay district: Cat Lake; Lac des Mille Lacs. Rainy River district: Mercutio Lake; Snodgrass Lake; rapid water between Saganagons and Kahnipiminanikok Lakes; lake west of West Lake and north of South Lake; Iron Lake; Knife Lake; Little Knife Lake.

Stagnicola lanceata is apparently widely distributed in western Ontario. The type locality is Pic Lake, north of Lake Superior. It was not included in the collections reported in 1931 (6).

Stagnicola cf. *nasoni* (F. C. Baker)

Rainy River district: Keats Lake.

A few specimens of a *Stagnicola* apparently referable to *nasoni* were collected in 1932 by Dr. Cahn. The colour is greenish-horn upon which the rest varices show as white vertical lines. The Knife Lake specimens are not exactly like the form found on the shores of the Great Lakes, and more specimens might show it to be a recognizable race. It is tentatively referred to *nasoni* at present.

Stagnicola emarginata kempi Baker and Cahn

Rainy River district: Basswood River; Kahnipiminanikok Lake; Carp Lake; McAree Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Kenora district (Lake of the Woods): McPherson Island. Minnesota: St. Louis Co., Iron Lake.

A further study of this race of *Stagnicola* has led the writer to relate it to *emarginata* rather than to *catascopium*, as indicated in the original description (6, p. 53, Plate II). It appears to be more intimately related to typical *emarginata*, but differs from that form in several particulars. This *Stagnicola* appears to be the dominant form of the genus in western Ontario. In Basswood River is it very abundant.

Stagnicola caperata (Say)

Rainy River district: Mack Lake.

Two specimens of this small lymnaeid were collected by Dr. Lindenborg in a swale near the lake.

Bulimnea megasoma (Say)

Thunder Bay district: Chief Peter Lake; Lac des Mille Lacs swamp. Rainy River district: Kashaboiwe Lake; Emerald Lake; Mowe Lake; Little Knife Lake; between Saganagons and Othermans Lakes; Athlestane Lake; Basswood Lake; Knife Lake; South Arm, Saganaga Lake; Baird Lake; Mercutio Lake; Snodgrass Lake; Keats Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Otterskin Lake. Minnesota: St. Louis Co., Iron Lake.

Bulimnea megasoma is very abundant in Ontario, where it appears to reach its maximum in development. Most of the specimens are brownish with a greenish tinge and the interior of the aperture is usually rich purple. Average specimens are about 45 mm. in length.

FAMILY PLANORBIDAE

Helisoma anceps (Menke) 1830 (= *Planorbis antrosus* Conrad, 1834)

Rainy River district: Basswood River; lake west of West Lake and north of South Lake (very abundant); Mackenzie Arm, Lake Kahnipiminanikok; Emerald Lake. Kenora district (Lake of the Woods): McPherson Island; Horseshoe Lake.

This species of *Helisoma*, long known under the name *Planorbis bicarinatus* and later as *Helisoma antrosus*, is very abundant in some of the lakes of Ontario. The material examined shows little variation from the typical form. In Emerald Lake, specimens had the wrinkled body whorl to which Currier gave the name *corrugatus*. Typical *anceps* were not recorded by Baker and Cahn (6).

Helisoma anceps sayi (F. C. Baker)

Rainy River district: lake west of West Lake and north of South Lake.

About 5% of the specimens in this lake are referable to the race distinguished as *sayi*, first noted in Wisconsin in Tomahawk Lake (1, p. 322). In this race the basal carina is near the peripheral edge of the whorl. In many lakes *sayi* only is represented, but in others it is mixed with typical *anceps*.

Helisoma anceps royalense (Walker)

Rainy River district: Carp Lake; Mercutio Lake; Camel Lake; Basswood River rapids. Thunder Bay district: Northern Light Lake.

The race of *anceps* called *royalense* was not common in the collections made more recently by Dr. Cahn. It was more abundant in the lakes in Thunder Bay district reported upon in 1931 (6, p. 55).

Helisoma anceps rushi Var. Nov. (Fig. 1)

In northern Minnesota and southern Ontario a form of *anceps* occurs that was previously referred to *jordanense* Winslow. An examination of material of *jordanense* from the type lot indicates clearly that the form living in Canada and Minnesota is not this race but one hitherto unrecognized. *Jordanense* (Fig. 1) is a Pleistocene fossil, the specimens being white and chalky. They were dredged from the bottom of Lake Charlevoix, Charlevoix Co., Mich., apparently from a marl deposit. *Lymnaea bakeri* Walker is from the same deposit.

Rushi differs from *jordanense* in the comparative lesser height of the body whorl behind the aperture, more rounded contour of the body whorl laterally, the less distinct carina at the edge of the whorl on the base and the rounded, not sharply angled margin of the spire depression. The recent race is also smoother and quite polished, while the sculpture of *jordanense* consists of strong, prominent lines of growth. *Rushi* is probably the living descendant of *jordanense*. Types of *rushi* are from Toad Island, Georgian Bay, Ontario, and were collected in two feet of water. Types, No. Z25259, Museum of Natural History, Univ. of Ill. Holotype: height 7.5; diameter 14.0; aperture height, 6.3; diameter 4.2 mm.

The new race has been seen from Minnesota and from the following localities in Ontario, Canada:

Rainy River district: Keats Lake; Kashaboiwe Lake; Fern Lake. Kenora district (Lake of the Woods): Otterskin Lake; Kennedy Island, near Whitefish Bay.

Helisoma trivolvis (Say)

Rainy River district: Athelstane Lake; Snodgrass Lake; Kahnipiminanikok Lake.

In the Baker and Cahn paper (6), *trivolvis* is recorded from Thunder Bay district as not uncommon. The specimens herein recorded from Rainy River district are fine typical examples. The species is widely distributed in western Ontario.

Helisoma trivolvis macrostomum (Whiteaves)

Thunder Bay district: South Arm, Saganaga Lake. Rainy River district: Kashaboiwe Lake; Mercutio Lake; Snodgrass Lake; between Saganagons and Othermans lakes; between Saganagons and Kahnipiminanikok Lakes.

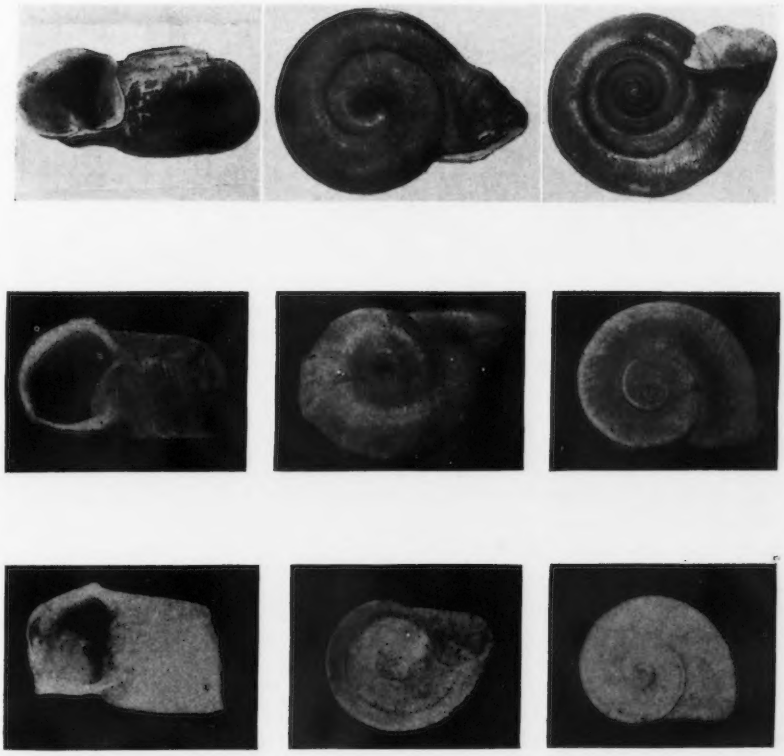

FIGURE 1.

Top Row. Helisoma campanulatum collinsi F. C. Baker. Cameron Lake, Lake of the Woods, Ontario. Left and middle figures, paratypes; right figure, holotype. Mus. Nat. Hist., U. of I., No. Z41451, Z41452.

Middle Row. Helisoma anceps rushi F. C. Baker. Toad Island, Georgian Bay, Ontario. Left figure, holotype; middle and right figures, paratypes. Mus. Nat. Hist., U. of I., No. Z25259.

Bottom Row. Helisoma anceps jordanense (Winslow). Lake Charlevoix, Charlevoix Co., Mich. Topotypes. Mus. Zool., Univ. Mich., No. 61589.

Figures enlarged 2.5 diameters.

This characteristic race of *trivolvis* is common in many parts of Ontario. The material in the collection now under study contains many large specimens 25 to 30 mm. in diameter. The race is well figured in the writer's discussion of *Helisoma corpulentum* (4, Plate 5).

Helisoma pilsbryi infracarinatum F. C. Baker

Rainy River district: Carp Lake; Knife Lake; Basswood Lake; Shebandowan Lake; Little Knife Lake; Mackenzie Arm and other parts of Kahnipiminanikok Lake; between Saganagons and Kahnipiminanikok Lakes.

The examination of a large series of this form of *Helisoma*, from a wide area of distribution, has convinced the writer that it is a race of *pilsbryi* F. C. Baker. Specimens from Ontario are usually quite characteristic and easily separated from *pilsbryi*, but in many lots there are specimens with a feeble basal carina indicating variation toward *pilsbryi*. Specimens from Rideau River, near Ottawa, Ontario, vary decidedly toward *pilsbryi*. *Infracarinatum* appears to be more widely distributed than *pilsbryi*. Both forms are figured by Baker (4). *Pilsbryi* is now believed to be a distinct species and not a race of *trivolvis*, as was stated (4).

Helisoma corpulentum (Say)

Rainy River district: Lake La Croix; Basswood River rapids. Thunder Bay district: Northern Light Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; McPherson Island; Kennedy Island. Minnesota: St. Louis Co., Iron Lake.

The finest specimens of this species came from Lake La Croix. These are large (27 mm. in diameter) and have the well-marked sculpture so characteristic of the species. The known records of this species indicate that it is common and widely distributed in western Ontario. See (4) for a discussion of *corpulentum* and its allies. It is interesting to note that one of Say's original localities for this fine species was Lake of the Woods.

Helisoma corpulentum multicostatum F. C. Baker

Rainy River district: Carp Lake; Mercutio Lake; Keats Lake; between Saganagons and Kahnipiminanikok Lakes; Kahnipiminanikok Lake. Thunder Bay district: Lac des Mille Lacs swamp. Kenora district (Lake of the Woods): Nestor falls, east of Sabaskong Bay.

Baker and Cahn (6) included this race under *corpulentum*. In the later paper (4) it is separated and figured. *Multicostatum* is as widely distributed as is typical *corpulentum*, often occurring in the same body of water.

Helisoma whiteavesi F. C. Baker

Thunder Bay district: Lac des Mille Lacs; Chief Peter Lake. Rainy River district: Athlestane Lake; Crooked Pine Lake; Basswood Lake; Carp Lake; Kashaboiwe Lake.

This fine *Helisoma*, first noted in Lac des Mille Lacs, is more widely distributed than was indicated in (4). The species is at once recognized by its great axial height, flattened spire, and few basal whorls. It is the climax of development of the *corpulentum* group.

Helisoma campanulatum (Say)

Rainy River district: Crooked Pine Lake; Carp Lake. Kenora district (Lake of the Woods): Horseshoe Lake, east of Kakagi Lake; McPherson Island; Beggs Lake.

Material apparently referable to typical *campanulatum* occurs in western Ontario. In some lots, as those from Beggs Lake, there is a tendency to vary toward the race called *canadense*. Only the race *canadense* was noted by Baker and Cahn (6).

Helisoma campanulatum canadense Baker and Cahn

Thunder Bay district: Northern Light Lake; Cat Lake; Lac des Mille Lacs. Rainy River district: Otter Track Lake; Kashaboiwe Lake; Carp Lake; Brent Lake; Mackenzie Arm and other places in Kahnipiminanikok Lake; lake near Mercutio Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; Kennedy and Russell Islands, near Whitefish Bay; McPherson Island.

This small race of *campanulatum* appears to be the dominant form of the species in western Ontario. It is described and figured by Baker and Cahn (6, p. 57). In many lots there is a tendency to vary toward the typical form of *campanulatum*, while in others the small race is quite distinct.

Helisoma campanulatum collinsi Var. Nov. (Fig. 1, top row)

A form of *campanulatum*, apparently abundant in the Lake of the Woods region, differs from all other variations of this species in the form of the spire, which is raised and forms a flattened dome, and in the larger number of closely coiled whorls, which total seven. The base shows 3½ whorls, and the umbilicus is small, round, and deep. The holotype measures: height, 6.0; major diameter, 15.7; lesser diameter, 12.8; aperture height, 5.5; aperture diameter, 4.2 mm. Types from Cameron Lake, northeast of Kakagi Lake (Lake of the Woods); Holotype Z41451, Paratypes Z41452, Museum of Natural History, Univ. of Ill.

This race of *campanulatum* bears some resemblance to *H. multivolvis* (Case) in the raised spire and tightly coiled whorls. The latter, however, has eight whorls, and the base is reamed out much as in *H. campanulatum rudentis* Dall. Also, the spire whorls in *multivolvis* are higher and give a mammiform appearance to the upper surface of the shell.

Besides the type locality, where the variety is very abundant, the new race has been seen from Otterskin and Shingwak Lakes, respectively east and northeast of Kakagi Lake, Lake of the Woods district.

This interesting race of *campanulatum* is dedicated to Dr. W. H. Collins, late Director of the National Museum of Canada. Dr. Collins was greatly interested in the development of the study of Canadian Mollusca.

Planorbula armigera (Say)

Thunder Bay district: Lac des Mille Lacs; Wauwiag River. Rainy River district: Snodgrass Lake; Kashaboiwe Lake; Reserve Arm, Lake Kahnipiminanikok. Kenora district (Lake of the Woods): Beggs Lake.

As previously noted (Baker and Cahn, 1931, p. 58), the Canadian material divides into two forms, one with the upper surface of the body whorl sharply carinated and the other with the body whorl rounded. The latter was named variety *palustris* in the paper referred to above, but its distinctness in large series of specimens may be doubted.

Gyraulus deflectus obliquus (DeKay)

Thunder Bay district: Wauwiag River. Rainy River district: Mack Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Kennedy Island.

This characteristic race of *deflectus* appears to be widely distributed in western Ontario.

Gyraulus latestomus F. C. Baker

Rainy River district: McAree Lake.

This recently described species of *Gyraulus* (2, p. 9) related to but distinct from *G. deflectus obliquus*, is at present known only from the type locality.

Gyraulus hornensis F. C. Baker

Rainy River district: Othermans Lake; lake west of West Lake and north of South Lake.

Much of the material listed in (6) is referable to *hornensis* rather than to *arcticus*, which is probably confined to Greenland. The records at hand indicate that *hornensis* has a wide distribution in Canada. See (3) for the description of this species.

FAMILY ANCYLIDAE

Ferrissia parallela (Haldeman)

Rainy Lake district: Otter Track Lake.

Common and variable in this lake. As special search must be made for these minute freshwater limpets, the few specimens found by Dr. Cahn in Ontario can scarcely be considered sufficient to indicate the distribution of the group in this area.

FAMILY PHYSIDAE

Physa gyrina Say

Thunder Bay district: Savanne River; Lac des Mille Lacs. Rainy River district: Lake La Croix; Snodgrass Lake; Emerald Lake; Kashaboiwe Lake; Keats Lake; Crystal Lake, north of Pickerel Lake; Reserve Arm of Kahnipiminanikok Lake. Minnesota: St. Louis Co., Iron Lake.

Most of the material from western Ontario is referred to *gyrina* by Dr. Clench. The specimens are small for the most part, scarcely exceeding a half-inch in length. One specimen from Snodgrass Lake is 19 mm. in length, indicating that the species does approach in size the *gyrina* of northern United States. Possibly much of the Ontario material is immature. All specimens have thin shells.

Physa sayii Tappan

Thunder Bay district: Mack Lake. Rainy River district: Mercutio Lake; Sark Lake; Emerald Lake. Kenora district (Lake of the Woods): Rabbit Point, northwest of Sabaskong Bay; McPherson Island.

Physa sayii is apparently widely distributed in western Ontario. The shell attains a large size (21 mm. in length) and is generally thinner than the shells found farther south.

Physa warreniana Lea

Rainy River district: Cub Lake; Sark Lake. Kenora district (Lake of the Woods): Otterskin Lake, east of Kakagi Lake.

The specimens collected by Dr. Cahn are apparently the first of this species to be listed from Ontario. The specimens are large, an individual from Sark Lake measuring 22 mm. in length. Although only beach specimens were collected by Dr. Cahn, the wide distribution of the species would indicate that it is common in western Ontario. Both *sayii* and *warreniana* occurred in Sark Lake.

Physa integra Haldeman

Kenora district (Lake of the Woods): McPherson Island.

A single bleached shell apparently referable to this species was found on the beach. (Identification by F. C. Baker.)

Aplexa hypnorum (Linn.)

Rainy River district: Mack Lake.

A few specimens of this widely distributed species were collected by Dr. Lindenborg.

Pulmonata

The pulmonate material listed was collected for the most part by Dr. Lindenborg in the Quetico Provincial Park, Rainy River district, Ontario. A few species were collected by Dr. Cahn. In the list the initials (C) and (L) indicate the collector.

FAMILY ZONITIDAE

Retinella binneyana (Morse)

Thunder Bay district: Wauwiag River (C). Rainy River district: Mack Lake; Agnes Lake; west end, Russell Lake; near Mackenzie Arm of Lake Kahnipiminanikok (L). Kenora district: Kennedy Island (C).

Typical specimens of this northern species of *Retinella*.

Hawaiia minuscula (Binney)

Thunder Bay district: Wauwiag River (C).

Only one specimen collected.

Striatura milium (Morse)

Rainy River district: Mackenzie Arm, Lake Kahnipiminanikok (L).

One specimen of this diminutive species was found under a log.

Zonitoides arboreus (Say)

Thunder Bay district: Shebandowan Lake; Wauwiag Lake (C). Rainy River district: near Lake Agnes; Mack Lake; west end, Russell Lake; Louisa Lake; Mackenzie Arm, Lake Kahnipiminanikok (L). Kenora district: Kennedy Island (C).

This species appears to be as common in Ontario as it is in the United States.

Euconulus fulvus (Müller)

Rainy River district: west end, Russell Lake; Mackenzie Arm, Lake Kahnipiminanikok; Mack Lake (L).

Only a few specimens of this cosmopolitan species were collected. They are similar to specimens from Minnesota and Wisconsin.

Vitrina limpida Gould

Rainy River district: west end, Russell Lake. Several typical specimens collected by Dr. Lindenborg.

FAMILY ENDODONTIDAE

Anguispira alternata (Say)

Kenora district (Lake of the Woods): Kennedy Island (C). Two shells. Rainy River district: between Louisa and Agnes Lakes (L). One specimen.

Discus cronkhitei anthonyi (Pilsbry)

Thunder Bay district: Wauwiag River; Shebandowan Lake (C). Rainy River district: Mack Lake; west end, Russell Lake; near Lake Agnes; near Mackenzie Arm, Lake Kahnipiminanikok (L).

The *anthonyi* from Ontario were found under conditions similar to those under which they live in central United States. The whorls are rounder than in *anthonyi* from some parts of the United States, and in some specimens approach *cronkhitei*.

Family Pupillidae

Columella edentula (Drap.)

Several specimens were collected from near the shore of Mack Lake, Rainy River district (L).

Vertigo modesta (Say)

Rainy River district: Mack Lake; near Mackenzie Arm of Lake Kahnipiminanikok; west end, Russell Lake (L).

One specimen from the first, two specimens from the second, and one specimen from the third locality.

Zoogenites harpa (Say)

Rainy River district: Mack Lake; west end, Russell Lake; near Mackenzie Arm, Lake Kahnipiminanikok (L).

This peculiar little mollusc appears to be common in Ontario. The specimens were found under logs near the camp ground.

Family Strobilopsidae

Strobilops labyrinthica (Say)

Rainy River district: Mack Lake, near Agnes Lake; west end, Russell Lake (L).

The specimens were found under logs and loose bark. Apparently common.

Family Cochlicopidae

Cochlicopa lubrica (Müller)

Rainy River district: Mack Lake (L).

Several specimens of this widely distributed species were collected.

Family Succineidae

Succinea ovalis Say

Kenora district: McPherson Island (Lake of the Woods) (C). One half-grown specimen. Rainy River district: near Agnes Lake (L). Two specimens.

Succinea grosvenori Lea

Thunder Bay district: Lac des Mille Lacs (C).

Two specimens were collected from debris.

Succinea retusa Lea

Thunder Bay district: Lac des Mille Lacs. Rainy River district: shore of Lake Kahnipiminanikok. Kenora district (Lake of the Woods): McPherson Island (C).

A few specimens were found on the shore of the lakes mentioned. *Retusa* and *grosvenori* were identified by Dr. Harold A. Rehder, of the United States National Museum.

FAMILY PHILOMYCIDAE

Pallifera dorsalis (Binney)

Rainy River district: Mack Lake (L).

One specimen of this slug was collected. It is like the form found in Michigan.

FAMILY LIMACIDAE

Deroceras cf *gracile* Raf. (*Agriolimax campestris* Binney)

Rainy River district: Louisa Lake; west end, Russell Lake; Agnes Lake (L).

A small slug, believed to be this species, was collected from logs near camps.

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PATHOGENICITY OF *BEAUVERIA BASSIANA* (BALS.) VUILL. ON COLORADO POTATO BEETLE LARVAE[1]

By M. I. Timonin[2]

Abstract

Beauveria Bassiana (Bals.) Vuill. isolated from infected Colorado potato beetle larvae is shown to be a pathogen to this host. The experiments indicated that some of the infected larvae may pass through the larval stage, but owing to further mycelial growth finally succumbed in the pupal stage. The fungus does not attack the eggs, but infected young larvae were found dying on the fourth day after hatching, when the eggs had been dusted with *B. Bassiana* spores. The mycelial growth on the infected larvae is more rapid in the soil than above ground.

Introduction

In September 1935, dead larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) were collected in a potato field at Fredericton, New Brunswick, and forwarded for examination to the Division of Botany by Mr. R. P. Gorham, Dominion Entomological Laboratory, Fredericton, N.B. The larvae were covered with soil particles, but a thin white mycelial growth, incrusted with conidial heads, dotted the surface of the larvae. The fungus was identified as a *Beauveria* sp. and was isolated in pure culture.

In 1935, Poisson and Patay (2) described a new species of the genus *Beauveria* pathogenic to Colorado potato beetle larvae and named it *Beauveria doryphorae*. Their description of the fungus may be summarized as follows: Culture white in colour, dense, velvety, forming a mycelial turf with a chalky appearance due to the abundant production of conidia; conidia perceptibly oval, 1.8 to 2.0 μ in diameter, and the phialides bearing them ventricose; phialides and conidia forming an aggregation about 30 μ in diameter; potato media not coloured red by the fungus.

The Canadian fungus, grown on potato agar with two per cent dextrose, is at first white, becoming light cream in colour later. Mycelial growth consists of a cottony or fluffy mass of hyphae, and frequently spreads over the glass in strands, filling the test-tube almost entirely within 10 to 15 days of incubation. Prophialides are ovoid to globose in whorls, bearing one or more, frequently two, phialides. Phialides are oval or flask-shaped with a long thread-like beak of zigzag shape. Conidia are globose or globoid, about 2.0 to 3.5 μ long and 2.0 to 3.0 μ wide. The reverse colour of the colony is cream buff and the colour of the medium remains unchanged. The conidia and conidia-bearing organs closely resemble those of *Beauveria Bassiana* isolated by the author from European corn borer larvae, but the latter isolate differed greatly in gross appearance owing to the abundant production of conidia in place of mycelium.

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If the above descriptions are compared, it is evident that the Canadian isolation differs from the European by the type of growth and the colour of mycelium, as well as in the shape and size of the conidia. A culture of the Canadian fungus was sent to Dr. C. Petch, who identified it as *Beauveria Bassiana* (Bals.) Vuill.

The purpose of this investigation was to determine whether this fungus is a pathogen of Colorado potato beetle larvae.

Material and Methods

With a view to securing sufficient spores of *Beauveria Bassiana* for field experiments, the fungus was grown on soybean mash in Petri dishes. The method used in the preparation of the mash, and propagation of the fungus was the same as that already described in a field experiment with *Beauveria Bassiana* (3). After four to five days of incubation, the fungus developed luxurious mycelial growth, but did not form spores as freely as isolates of *B. Bassiana* from corn borers on the same medium.

In order to test the pathogenicity of *Beauveria Bassiana*, several potato hills, naturally infested with Colorado potato beetle larvae, were selected in the experimental potato plots and caged with cheese cloth.

The larvae were dusted with the conidia by dipping a small camel's-hair brush into a culture of the *Beauveria* and gently tapping it while it was held over the larvae.

Pathogenicity of *Beauveria Bassiana*

On July 15th, 1936, four potato hills were caged with cheese cloth, the larvae counted and in three cages they were dusted with *Beauveria* spores. The fourth was kept as a check. The larvae at that time were in the prepupal or in the last larval instar stage. The results of this experiment are summarized in Table I.

TABLE I

PATHOGENICITY OF *BEAUVERIA BASSIANA* ON COLORADO POTATO BEETLE LARVAE

Cage No.	No. of larvae dusted	No. of larvae infected 3 days after dusting	No. of dead larvae found 6 days after dusting	No. of dead larvae found in the soil 14 days after dusting	No. of pupae found in the soil	No. of pupae with brown marks and mycelial growth	No. of pupae with healthy appearance	Per cent of survival
1	21	18	7	8	5	3	2	9.5
2	19	19	5	12	2	2	0	0
3	25	21	9	8	8	4	4	16.0
4	20 untreated (check)	None	None	1	19	None	19	95.0

On the third day after inoculation, brown to dark brown lesions were observed under the epidermis of the infected larvae (Fig. 1), while at the same time uninoculated larvae in the control cage remained normal. Larvae

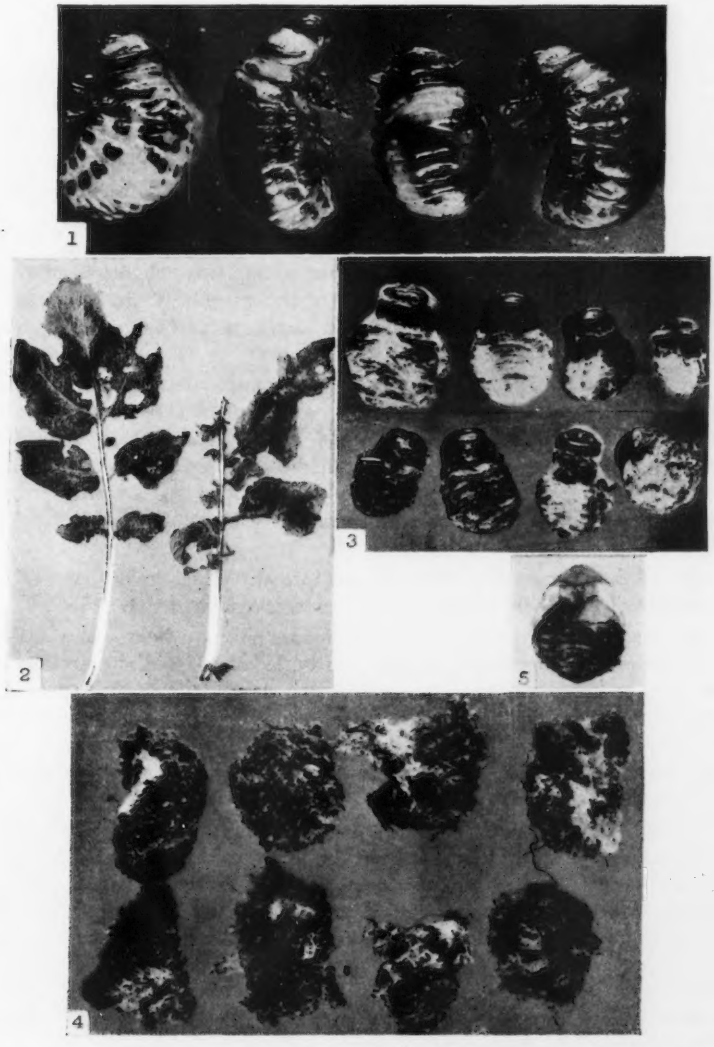

FIG. 1. *Larvae four days after dusting. Note the dark brown lesions of infection.* ×3½. FIG. 2. *Mummified larvae attached to the leaves.* ×¾. FIG. 3. *Mummified larvae found on the ground.* ×3½. FIG. 4. *Mummified larvae, surrounded with soil particles, from soil under dusted plants.* ×2. FIG. 5. *Pupa with dark lesions of infection.* ×2.

bearing such lesions were somewhat less active and did not feed as freely as the larvae in the control cage. When the lesions reached a diameter of 2 to 3 mm. (Fig. 1, second larvae from the left) the larvae became practically motionless and died shortly afterwards. The interiors of the dead larvae were completely filled with mycelium and some mycelial growth developed on the surfaces of the larvae.

This observation is somewhat similar to that reported by Boczkowska (1). Studying immunity of the larvae of *Galleria mellonella* L. to entomogenous fungi, she observed that caterpillars infected with the entomogenous fungi often developed black spots under the chitin. These black spots were found to contain agglomerations of leucocytes. She also noticed that larvae covered with lesions 1 to 2 mm. in diameter always succumb, whereas those with delicate spots sometimes survive and pass through metamorphosis.

Only a few dead larvae remained attached to the leaves of the potato plants (Fig. 2); the majority of the larvae fell to the ground. Dead larvae which were collected on the ground were covered with more surface mycelial growth than those which remained attached to the leaves (Fig. 3).

Metamorphosis of the larvae occurs below ground. Probably because conditions were more suitable for the fungus growth, many of the infected larvae that entered the soil became mummified after three to four days. The mycelium spread in all directions through the soil particles and bound them together, completely surrounding the larvae in a layer of soil which formed a soil ball 1 to 1.5 cm. in diameter incrusted with white mycelial threads (Fig. 4).

Some of the larvae successfully passed into the pupal stage, but the dark infected areas could still be seen on the epidermis of the pupae (Fig. 5).

Two flower pots were filled with greenhouse soil and in one four healthy, and in the other four infected, pupae were buried about 2 in. deep. The pots were kept in the greenhouse, and within four days of incubation four adults emerged from the healthy pupae. The second flower pot, containing the infected pupae, was examined on the fourth day of incubation, when all four pupae were found to be mummified and to have the same appearance as the larvae in Fig. 4.

The daily temperatures, humidity of the air, and rainfall during the period of this experiment, have been obtained through the courtesy of the meteorological station at the Central Experimental Farm, Ottawa. Taking into consideration these data, we may conclude that the climatic conditions prevailing were favourable to the fungus growth. The highest temperature during the experiment was 83° F., which is around the optimum for fungus growth; the humidity of the air, according to the two daily readings, was sufficient for mycelial growth, and the few rain showers kept the soil sufficiently moist for the rapid growth of the fungus.

This experiment was repeated several times when larvae and potato plants were dusted with *Beauveria* spores; but plants were not caged in cheese cloth,

and it was impossible to estimate the number of larvae actually involved in the experiment, owing to the fact that larvae were continually emerging from the newly laid eggs. Nevertheless, observations indicated that the fungus does not attack the eggs. However, infected young larvae were found dying on the fourth day after hatching, when the eggs were dusted. It would therefore appear that at any stage of their development the larvae are susceptible to *Beauveria Bassiana* isolated from this host.

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